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THE SARCOSPORIDIA. A CRITICAL REVIEW*

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Sarcosporidia were first discovered by Miescher in the muscles of mice in 1843. Since that time many observations and experiments have been made, and much has been written about these organisms, but still our knowledge of their life history is very incomplete. Many contradictory opinions have been expressed concerning these parasites, divergent results have been obtained in their study and erroneous conclusions drawn, frequently from inadequate data. Under these circumstances, it is not out of place to sum up and scrutinize carefully our present knowledge of the group.

Sarcosporidia are parasitic organisms of uncertain relationship and as yet undetermined biological position. They infest the muscle tissue and occasionally the connective tissue of mammals, birds, and reptiles. They have been found chiefly in mammals of herbivorous or omnivorous habits, in a considerable number of birds and in a few reptiles. A few cases have been reported for man but these are regarded as accidental infections. Sarcosporidia frequently infest the muscle tissue in enormous numbers as in the sheep, pig, ox, mouse, and some birds. In some domestic animals, as in the sheep, pig, ox, and horse, they apparently have a world-wide distribution. Consequently, from the economic standpoint Sarcosporidia are of chief interest to the veterinarian rather than the physician.

Ray Lankester established the genus Sarcocystis in 1882. Some forty-four or more species of Sarcosporidia have been described, and all have been assigned to this genus with one exception. Hadwen (1922) has named a form found in the reindeer and caribou *Fibrocystis tarandi*, on the distinction that it was found in fibrous connective tissue, especially of the tendons and of the periosteum next to the bone surfaces. The various species of Sarcocystis have been distinguished chiefly on the basis of the host infested, the size of the cyst or of the spores, or of some other seemingly insignificant differences. In view of the further fact that transmission of a species to a new host results in altering the size of the parasite, as reported by Darling, Erdmann, Betegh and

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Dorcich, and others, it has been suggested by Alexieff (1913) that only one species exists. There is much evidence against this view, however, for transmission to a foreign host is not easy to accomplish.

The relation of Sarcosporidia to other organisms is likewise uncertain. They have been frequently classified with the Cnidosporidia, but they have none of the principal characteristics of that group. So little is known of their life history and biological relationships that they may not even belong to the Sporozoa. Wenyon (1926) has called attention to their close relationship to Globidium which Ashworth (1923) has shown is one of the fungi. The development of the sarcocyst in muscle tissue is well known but on the remainder of the life history, how the parasite passes from the muscle of one host to that of another and the changes it undergoes, facts are fragmentary, uncertain, and in part entirely unknown.

The discovery of truth involves certain important principles of scientific method. In scientific procedure there is the need of rigidly accurate observations, carefully planned experiment, and logical reasoning in drawing conclusions. A critical study of the development of knowledge of the Sarcosporidia reveals that one and all of these principles have from time to time been violated. Such procedure leads to confusion of ideas, contradictory results, speculative hypotheses and erroneous conclusions. While not claiming infallibility, this paper is an attempt to sift out the main facts from the great mass of work that has been done on the Sarcosporidia.

For nearly fifty years after the discovery of Sarcosporidia most of the work on the group had to do with observations on nine or ten of the most common species, their occurrence in different locations and tissues of the host, the per cent of animals infected, the intensity of the infestation, the size of the Miescher's tubes, especially of the Balbiania, the presumed pathogenicity of the parasite, and the diseases attributed to it. The papers of Manz, Railliet, Moulé, Morot, and Bertram represent some of the best work of this period. Some accurate observations were made and the morphology of the sarcocyst and of the spores was fairly well worked out.

In 1890 Pfeiffer was the first to experiment with the effects of injected emulsions, using *Sarcocystis tenella* for this purpose. This work opened up a new method of investigation which led to the discovery of "sarcocystin" and various experiments on the pathogenicity of the parasites which will be referred to later. Piana (1896) undertook to culture the spores but his results were inconclusive. Smith (1901) was the first to transmit Sarcosporidia by feeding infected muscle to uninfected mice. This method of study has led to a long series of experiments that have given in a few instances some of the most impor-

tant information in regard to the life history and development of these parasites. Bergmann (1913) made extensive observations on the development of sarcocysts with reference to the age of the host, per cent infected at different ages, the earliest age at which animals were found infected, the time of year at which invasion of muscles occurred, and location of the parasites in the body. Numerous other workers have extended these observations. Many studies have also been made on the morphology and development of the muscle stages and various methods of good, indifferent, or poor technique have been employed in the study of both fresh and preserved material.

I have thus indicated briefly the general nature of the work that has been done on the Sarcosporidia, and shall now take up in more detail the study under three main headings: (1) morphology and development of the sarcocysts, (2) life history, and (3) host-parasite relations, including certain transmission experiments, pathogenicity, and host specificity.

MORPHOLOGY AND DEVELOPMENT OF THE SARCOCYST

Moulé (1886) showed that infection is generalized in the striated muscles. For a study of the muscle stage the beginner should look for Sarcosporidia in the esophagus, heart, diaphragm, masseter and tongue muscles, and at first examine older animals, especially those that are in poor flesh, or emaciated, or that have been kept for some time under crowded pasture conditions. Bergmann (1913) observed that Sarcosporidia in calves and lambs appeared earliest and most abundantly at the lower end of the gullet; that they rarely appeared in these animals before 10 weeks and never before 6 weeks of age. He also noted that they were not found in the smooth muscle of the stomach only four centimeters from the lower end of the esophagus. Considering this well marked specificity for striated muscle tissue it is a rather curious fact that Sarcosporidia have been found in the lips, in the skin, in the submucous connective tissue of the intestine, and in the dura mater of the brain.

The size of the sarcocyst depends upon internal factors, such as growth and reproduction of the spores, and external factors, such as compactness or looseness of the enclosing tissue, and the nutrition available. The shape of a sarcocyst depends upon its location in the body, its age, and the nature of the enclosing tissue. For example, mature cysts in the diaphragm are much elongated and spindle shaped. In heart muscle of the sheep, the younger cysts are usually circular in cross-section and elliptical in longitudinal section; in early stages the length is several times the diameter, but later the diameter increases proportionately faster than the length. Some cysts are very irregular in outline, probably due to unequal resistance to growth pressure from within. Possibly such cysts are on the point of disintegration.

The development of the sarcocyst in the muscle is well known and runs a more or less uneventful course from the time a sporoblast cell enters a muscle fiber until by growth and reproduction a large sarcocyst is formed that may contain millions of spores. In this development there is both an intra- and later an inter-cellular stage. The sporoblasts become transformed into young spores, and both sporoblasts and spores increase by cell division. According to Fantham and Porter (1914) the cyst membrane may rupture at an early stage, allowing the sporoblasts to escape and invade other muscle fibers. My own observations indicate that this rarely if ever occurs in *Sarcocystis tenella*. Methods of micro-technique show that the internal network as well as the cyst wall of Balbiania are both derived from the host.

The earliest stage of development in the muscle fiber seems to have been seen by Crawley (1916), who observed a single partly divided "sporoblast" of *S. tenella* lying in a minute cavity within a cell of the heart. The next stage found consisted of eight sporoblasts, and Crawley thinks it comparable to the earliest stages of *S. muris* described by Bertram (1892) and Negri (1910). Marullaz (1920), after feeding mice with infected material has found in the muscles two and eight-celled stages which he regards as young stages of *S. muris*. According to some authors, Negri and Fantham and Porter, the youngest stage in the muscle develops into a multinucleate plasmodium enclosed in a membranous sheath; segmentation of the mass occurs and the membrane may rupture, allowing the sporoblasts to escape and invade other muscle fibers; or the sarcocyst may continue to increase in size as the result of division of the sporoblasts and spores. Negri (1908), Betegh (1909), Fiebiger (1910), Fantham and Porter (1914), and others find that the young spores reproduce by longitudinal fission. By growth the parasite may completely obliterate the host cell and come to lie in connective tissue. Balbiania is now regarded as a much enlarged sarcocyst that has grown far beyond the limits of the original muscle fiber. In sheep they are usually limited to the muscles of the esophagus, larynx, pharynx, tongue and palatal regions, though occasionally they may be found in other regions. Their location and development is probably related to the nature of the tissue in which they are found. A careful study should be made to determine the factors controlling their limited distribution.

A great deal of work has been done on the morphology and development of the sarcocyst, and particular attention has been given to the structure of the cyst including the cyst wall, and the morphology of the spores. Such information the reader can obtain from Alexieff, Bertram, Betegh, Erdmann, Fiebiger, Schneidemühl, Teichmann, Wasielewski, Wenyon, and others. The limits of this paper will not permit

an extended review of their work. While they differ in some details, the main facts are well established as outlined above.

Probably this is the place to say something of the methods of study employed. In going over the literature one is impressed with the idea that the results obtained have been largely determined by the methods of technique used. Especially is this true of fixed or preserved material. Sarcocysts can be detected by the naked eye only in the older stages, either as Balbiania or as large cysts that so completely infest the tissue that they give it a streaked or abnormal grayish appearance. Necessarily, microscopic methods have been employed for both fresh and fixed material. A simple method of examining fresh material is to take a small piece of muscle, crush it between two slides or under a cover glass, and examine it directly under low powers of the microscope. Stains, such as methyl green, which have an affinity for fresh tissue are sometimes employed. Fresh muscle may be teased apart in acetic acid tinged with thionin; or, it may be fixed for a short time in 10 per cent formalin, frozen, sectioned, and stained in thionin or haemalum-eosin. By these or other relatively simple methods one may detect medium or large sized cysts, determine their numbers per weight or volume of muscle present, and observe the lobulated appearance of the sarcocyst as a whole.

Koch (1902) macerated cysts from a dead mouse in physiological salt solution and observed movement of the spores, when heated somewhat above blood temperature in a thermostat on the stage of a microscope. Betegh (1909) worked on fresh Sarcosporidia, partly with dark field illumination, partly with permanent preparations; the latter were stained with Giemsa, Romanowsky, or Löffler's methylen blue after fixation in ethyl or methyl alcohol. For dark field illumination the cysts were freed from the muscle fibers, cut open with a sharp razor, the contents transferred with a platinum needle to a slide and the spores studied directly in lymph from the cyst. He noted a longitudinal contraction of that part of the spore that has no chromatin. Betegh found that if cysts were broken open in physiological salt solution and spread between two slides, the procedure of Negri, less satisfactory results were obtained; that is, the spores were deformed, with no clearly determined structure. The breaking up of spores into granules, as reported by some workers, is probably due to poor technique.

The writer has used the method of Betegh combined with the hanging drop method for the examination of fresh spores. The cyst is opened with a sharp razor, the contents are spread loosely on a cover glass, and the cover glass immediately inverted on a hollow ground slide. By this method the fresh spores may be observed with high powers of the microscope, in their natural medium, without disturbance

of their form or structure. If it is desired to keep the preparation under observation for some time, a little moisture may be placed in the cavity of the hollow ground slide and the cell sealed. If kept at room temperature, the cell may be sealed with vaseline; if kept at the temperature of the body, or higher, it is better to seal the cell with a light wax or cement.

For fixed preparations one may use dried films stained with Minchin's modification of Giemsa or other Romanowsky stains, but such preparations do not always give satisfactory results. The frequent disagreement in regard to the structure of spores is no doubt in part traceable to such methods. Betegh cuts open the cyst with a razor and immediately spreads the contents out in a thin layer on a cover glass. The "sporozoites" are not further disturbed by placing on a slide or other cover glass before fixation. Good photomicrographs were obtained from such preparations.

For a study of the structure of the cyst, the structure of the cyst wall, and for all the early developmental stages, thin sections must be prepared. Tissues are frequently fixed in picro-sublimate-acetic, picro-acetic, or Schaudinn's picro-sublimate mixture. Absolute alcohol, Flemming's fluid, Zenker's fluid, and many others have been used with varying success. Numerous stains have likewise been used. Heidenhain's iron-hematoxylin, hematoxylin and eosin, Romanowsky stains, and various other stain combinations are useful. Satisfactory technique varies with the time and method of fixation, the thickness of the section, the stain or stains used, and the care with which the sections are cut, cleared and mounted. It is perhaps needless to add that the tissue should be fixed as soon as possible after the death of the host.

Although the morphology and development of the sarcocyst stages of Sarcosporidia have received a great deal of study, a number of problems remain to be solved. The early stages of the parasite in the muscle fiber are still incompletely known. More information is needed about the cytology of the sporoblasts and the spores, including a study of their method of division. A study of the comparative morphology of the spores of so-called different species should be made, using tested and standard methods of fixation and stain technology. It would also be interesting to know under what conditions dissolution of the cyst takes place.

THE LIFE HISTORY

Our knowledge of the life history of the Sarcosporidia outside of muscle tissue is fragmentary. In regard to natural transmission something is known of the age and time of year at which some animals are infected, but there is no definite knowledge of how to cultivate the spores; the probable methods of natural infection are much in dispute,

and one finds only scanty knowledge of the viability of the spores under laboratory conditions. It appears that the spores are sometimes found in the blood and this fact may prove to be of considerable significance. Outside of the muscles, the chief knowledge of the life history is based on positive results of experimental infections, on some negative results that have helped to define the limits in which transmission can occur, and on direct observation. There is, however, still much disagreement over what are the actual facts, and there is so much missing that any interpretation of the facts in terms of the life history is only tentative. Such facts as are available fall naturally into two groups, one group related to the muscle stage, the other to experimental infections by way of the alimentary canal. The following account is a provisional attempt to piece out the life history and suggest some of the gaps that ought to be filled.

I have already traced the development in the muscle which results in the formation of "Rainey's corpuscles" or spores; let me now mention briefly some work on the spores that indicates their viability under laboratory conditions. A number of observers have noted movements of spores. For example, Pfeiffer (1890) saw spores of *S. miescheriana* stretch out in a spiral with a short radius, but observed no distinct locomotion. Koch (1902), working with *S. muris,* saw a spiral rotation of a spore about its long axis. Crawley (1914) notes that spores of this species are capable of very active twisting and boring movements. Betegh (1909) noted a longitudinal contraction of that part of the spore of *S. tenella* that has no chromatin, but no locomotion was demonstrable. I have seen such movements of this species upon several occasions, and in one instance observed pronounced locomotion. The tongue of an old ewe was kept for a week at a temperature that ranged from 15 to 20° C. On the seventh day a large cyst was removed from the deep muscles of the tongue, cut into halves, and one half was placed on a slide moistened with sterile normal salt solution. After mixing the contents of the cyst with the liquid, the mixture was transferred by means of a pipette to a cover glass for a hanging drop preparation. Both bacteria and spores were present; the bacteria exhibited Brownian movement, and the Sarcosporidia showed slight traces of motility. The slide was kept at room temperature. The next day the bacteria were much more numerous, the rod shaped forms were motile and the round forms showed Brownian movement. The Sarcosporidia were nearly all motile, would turn over, straighten out and move one-third the way across the field in a short time; they would twist and turn and change position almost constantly. The hyaline end of a spore would lengthen out, causing the spore as a whole to assume a long spiral shape, then as it contracted and resumed the normal or typical shape the spore moved forward a distance sometimes almost equal to its full length.

Twisting and turning movements, resulting in moderate changes of position, were more characteristic than progressive locomotion. Experienced co-workers, one a botanist and another a bacteriologist, both agreed that we had a true case of movement, and locomotion unlike anything they had seen. On the third day, the spores were inactive and apparently dead. One can only guess as to the significance of such movements. An important problem would be to learn how to culture the spores and study their further behavior.

The age at which young animals become infected under natural conditions is related to the life cycle of the parasite. Pfeiffer (1891) observed that young sheep have cysts, but sucking lambs do not. Bertram (1892) found only small cysts in an 8 months lamb, but no cysts in embryos. Bergmann (1913) made careful and extensive observations covering a long period. The youngest age at which he reports infection was as follows: lamb, 6 weeks; calf, 6 weeks; pig, 10 weeks. Infection was rare under 10 weeks and was not common until the animals were older. Of the colts examined, the youngest infected was 10 months old. McGowan (1914) examined eight lambs under 30 days of age and found no sarcocysts; of 121 February lambs, killed between April 30 and June 11, only 4 were infected. Walker (1918) found Sarcosporidia in a lamb 6 weeks old. Viljoen (1918) reported no infections in cattle embryos, or in a 2 months calf, though almost all cattle over one year had parasites. Scott (1918) was unable to find Sarcosporidia in sheep embryos, or in lambs under 10 weeks, though only a small number of lambs under 10 weeks of age were examined. Evidently transmission does not take place in the uterus, as suggested by McGowan.

It is interesting to compare the above facts concerning natural infection with the time it takes the parasite to become established in the muscles after experimental infection. Smith (1901) found Sarcosporidia in the muscles 45 days after feeding infected muscle. The result has been confirmed by Koch (1904), Nègre (1907), Crawley (1916), Marrulaz (1920) and others. Nègre got similar results after feeding food contaminated with feces from infected mice. Erdmann (1910) claims to have found the parasite in the muscle in 28 to 30 days after feeding infected muscle. Comparing these results with the age of natural infection, it is almost certain that natural infection and transmission take place after birth. While it may not be impossible for infection to occur in the uterus, McGowan's (1914) theory so far does not appear to have a single fact to support it.

A number of observations have been made on the time of year at which infection takes place. Pfeiffer (1891) states that the youngest cysts are found in the spring. Bergmann (1913) from data collected on sheep, cattle, swine and horses, concludes that invasion of Sarco-

sporidia reaches its greatest extent in spring and summer. Estimating 30 to 45 days for the parasite to reach the muscle, Scott (1918) demonstrated a well marked seasonal infection of lambs in Wyoming; by taking careful measurements of sarcocysts, he was further able to prove successive seasonal reinfections. This result agrees with the well known fact that, in older animals, the infection is heavier, and the number infected frequently rises to 100 per cent.

Another fact that undoubtedly bears some relation to the life cycle is the evidence for the dissolution of the mature sarcocyst. The evidence is both direct and circumstantial. Alvarez (1926) found indications that the cyst ruptures in the pig. Basing his conclusions on measurements of sarcocysts from lambs and sheep of known ages, Scott (1918) found that while the current seasonal infection of two year olds was apparently the same as for lambs of the same season the number of parasites remaining from the first (second preceding) infective season was much less than the number of parasites that definitely belonged to the current infective season. This same principle applied also to three and four year old sheep. Perhaps of even more significance is the fact that *Sarcocystis* spores have been found in the blood. Probst (1910) noted that he found spores in the heart blood of a calf. Castellani and Sturgess found them in the blood of *Bos indicus*. Sergent (1921) found them in a drop of blood secured by piercing the skin of the jaw of a calf, though many weekly examinations both before and after this date showed nothing. Arai (1925) found spores in the blood of all mice in which ripe Sarcosporidia were present in the muscles. The writer after a prolonged search has found spores of *S. tenella* in the blood of old ewes. Under these circumstances the presence of spores in the blood does not appear to be accidental, and is probably related to disintegration of the sarcocyst for which there is considerable evidence. Their presence free in the blood suggests that they are on their way to the outside of the host.

Some attempts have been made to cultivate the spores. Piana (1896) and Galli-Valerio (1916) reached inconclusive results. The writer also has made some unsatisfactory attempts in this direction. Here is an open field for investigation that should prove interesting.

Nearly all successful transmissions of Sarcosporidia have been the result of feeding experiments. Darling (1910) transmitted Sarcosporidia from the opossum to the guinea pig by intra-muscular inoculation but Nègre (1907) failed to inoculate mice with *S. muris*. In contrast, numerous workers have been able to infect both normal and foreign hosts by feeding muscle containing sarcocysts or by giving food contaminated with feces from infected animals. The results of Smith (1901, 1905) in producing infection of mice by feeding infected muscle have been confirmed by Koch (1904), Nègre (1907), Negri (1910),

Erdmann (1910) and Crawley (1914). Nègre (1907 also demonstrated experimentally an infective stage of *S. muris* in the intestine. He found that the feces of mice, which are infected by eating muscle, have the power to infect well mice from 15 to 60 days after they were fed the infected muscle. Crawley (1916) confirmed these results of Nègre with the added observation that infections by means of food contaminated with feces were heavier than those produced by feeding muscle. Scott (1918) isolated four young lambs at an early age in March and penned them together during the summer; he produced three very heavy and one average infection in these lambs by feeding to one of them grass that had been contaminated with feces from infected ewes. It is also true that *S. muris* and *S. tenella* have been transmitted to a number of foreign hosts by feeding muscle. *Sarcocystis muris* was transmitted by Negri (1908) to the rat and to the guinea pig, by Negri (1910) from the rat to the guinea pig, by Darling (1910) to the guinea pig. *Sarcocystis tenella* has been transmitted by Erdmann (1910) to the mouse, by Betegh and Dorcich (1912) to a hen and two ducks, and by Arai (1925) to the mouse. On the other hand, Pfeiffer (1891) states that butchers' dogs remain free from Sarcosporidia, though fed freely with meat containing enormous numbers of sarcocysts, including Balbiania. Scott (1925) did not succeed in infecting the white rat with *S. tenella* and he has repeatedly failed to infect lambs by feeding Balbiania or infested muscle.

The results of the transmission experiments suggest that infection in nature takes place by way of the alimentary canal. Indeed a number of observations have been made that support the experimental evidence given. Mason (1910) noted that in camels mature sarcocysts were most numerous in the esophagus and heart. Bergmann (1913) found in calves and lambs as noted above that Sarcosporidia appeared earliest and were most abundant at the lower end of the gullet. Schlegel (1920) states that observations on young goats show the tonsils to be the chief portal of entrance. Chiwy and Colback (1926) from a study of cattle in the Belgian Congo conclude that infection apparently takes place by the alimentary tract. Scott (1920a, b) raised lambs with their ewes in a screened cage which was kept free from carnivorous animals (mice, dogs), sheep ticks, and all other insects except a few small transient gnats; the lambs became more heavily infected than control lambs kept outside of the cage in a dry lot. He concluded that a second host was not necessary to transmit *S. tenella.* Scott and O'Roke (1920) were not able to infect lambs by feeding hundreds of the insects that appeared to be the only possible carriers of the infection if Darling's (1915) theory was correct. In the experience of the writer lambs raised under crowded conditions become more heavily infected than those that have a wider range

whether in pasture, dry lot, or screened cage. Considering all the facts, there is scarcely any doubt that food contamination is the usual method by which Sarcosporidia are transmitted to new hosts. As a matter of fact the work of Nègre and Crawley has demonstrated in the intestine of the mouse a resistant stage that is capable of infecting other mice. Continuing this work, Nègre (1918) states that the resistant stage appears 15 days after ingestion of sarcocysts and disappears by the seventy-fifth day; that the maximum power of infection is from the 20th day to the 50th day. According to Nègre, this resistant stage withstands desiccation for a month, and heating for 30 minutes to 60° C. does not destroy its infective power.

How the Sarcosporidia reach the muscle from the intestine is still largely unknown, and such facts as one finds are in dispute, or need further confirmation. Erdmann (1910) states that in the intestine the spore sets free a small amoeboid form which penetrates the epithelial cells. In various parts of the gut wall, lymph tracts and fat tissue, the parasite undergoes multiplication for a few days, and then disappears. Some 28 or 30 days later the parasite appears in the muscle. Her figures 1 and 2 of Plate XVII show spores apparently entering the epithelium, but the very much smaller amoeboid forms shown in figures 3, 4 and 5 do not have any resemblance to the spores of Sarcosporidia. Later (1914) she hesitates to say that these small amoeboid and schizogonic forms were derived from or connected with the spores of the Sarcosporidia that were fed. Crawley (1916) made an extensive study of the early stages of development of the spore in the intestine of the mouse. Upon reaching the lumen of the intestine, the nucleus of the spore becomes larger and the granules fade or disappear. He believed the spores are sexually differentiated into male and female forms, not distinguishable at first, and upon penetrating the epithelial cells undergo further sexual differentiation. He assumes that fertilization takes place, and the zygote proceeds to multiply in the villi. Since Crawley's interpretations have been questioned by Wenyon (1926) and were not in entire accord with Arai (1925), the writer through the kindness of Dr. Maurice C. Hall, Chief of the Division of Zoology, Bureau of Animal Industry, was permitted to examine Crawley's original preparations. The preparations clearly showed spores penetrating the epithelial cells, and that changes took place in the spores both before and after they penetrated the epithelium. Whether these changes were developmental or were degenerative in character, as suggested by Wenyon, I am unable to say. They appeared to be degenerating nuclei. A full series of drawings was prepared which included all stages found. There was no break in the series, and so far as could be determined, there was no sexual differentiation of the spores. In particular I was unable to find the so-called female spores.

Artefacts of the parasite, similar to Crawley's drawings of female spores were seen when focussing on the nucleus of the host cell, provided the parasite was in a slightly different focus.

Marullaz (1920) agrees with Crawley that the spores may be found in the epithelial cells within two hours. According to this author the form of the parasite then becomes rounded, the nucleus loses its granular structure, the chromatin becomes organized, and mitosis is begun; the parasites then pass into the lymphatic spaces, and complete division takes place during the time of their passage from the intestine to the liver and spleen where their products are found. The daughter spores then go to the striated muscles where they may be found from the 44th to 55th day. These cells in the muscles have one or two nuclei and in one case there appeared to be eight cells which the author regards as the beginning of a sarcocyst. Another group of spores becomes fixed in the epithelial cells, undergoes complete mitosis, and the products of division, either set free or still enclosed in the host cell, fall into the lumen of the intestine where they continue to multiply for a time, attaining their maximum number about the tenth day. Marullaz did not observe anything approaching the fertilization of spores as described by Crawley. As Wenyon points out, it is an exceedingly difficult thing to trace such minute objects in their wanderings about the body and not confuse them with other structures. The writer has seen cells, which were possibly parasites, in the epithelial and sub-mucosa layers of the intestine of the white rat that very much resembled the figures of Marullaz, but which were certainly not Sarcosporidia.

Arai (1925) infected the mouse with *S. tenella,* but according to this author infection is not so certain as previous workers would lead one to expect; the material must be fed fresh, less than five hours old. Two hours after feeding the spores are found in smear preparations along the entire small intestine and in the beginning of the large intestine; using a one per cent water solution of eosin to test the viability of the spores, it was found that the morphology of the spores in the intestine was unchanged, but those in the cecum or large intestine showed unfamiliar structure. Four hours after feeding all spores were changed in structure and evidently were in a degenerating or dying condition; the farther back the spores were, the more changed they were, and Arai believes that Crawley has described these same form changes as normal development. Sections of the anterior part of the small intestine taken two hours after feeding, not infrequently show spores passing between the epithelial cells or in the sub-epithelial spaces. He found no intra-cellular stages. Four hours after feeding and later, no spores were found in the gut tissue. Arai also found spores of *S. muris* in the heart blood of a mouse six hours after experimental feeding; in blood from the tail of another mouse he found spores five hours after

feeding, but none from the same mouse before or after this period. Between this time and the appearance of sarcocysts in the muscles 35 to 50 days later, the spores were not traced. The micro-photographs of Arai are convincing and it seems certain that the spores of *S. tenella* penetrate the intestinal epithelium of the mouse and reach the blood stream. The writer has fed Balbiania to young white rats and recovered some of the spores in the blood four and one quarter and six hours after feeding. In shape and staining reactions the spores appeared unchanged. Possibly this may be the normal method of entrance, or perhaps simply a transfer made possible by locomotive powers of the spore.

From this general account of the life history it would appear that much experimental work needs to be done in order to confirm or disprove some of the work already accomplished. The largest gap to be filled lies between the time the parasite penetrates the intestinal epithelium and its appearance in the muscle. How the spores reach the outside of the body from the blood stream is another problem. Is there a resistant spore stage to be found in the feces? If so, what is the fate of the spores outside of the host? The theory that the Sarcosporidia are transmitted by blood sucking insects does not seem to have any direct evidence to support it. Scott (1920) was unable to prevent infection in lambs by confining them with their ewes in screened cages, from which all biting insects were excluded. The parasite that Missiroli (1928) found in the muscle of *Anopheles maculipennis* evidently does not belong to the Sarcosporidia. The theory of Mrowka (1925) that the nucleus of the "spore" breaks up into very small nuclei or granules, and that this represents a method of reproduction and development of the spores is based solely upon slide preparations. Since the methods he used in preparing the slides frequently produce morphological changes in the spores, he is apparently dealing chiefly with artefacts. The supposed methods of development of spores, and their spread in the muscle and by the milk, as suggested by McGowan (1914, 1923), is based upon similar preparations. Interpretation of processes from morphological findings is an extremely difficult thing for the microscopist to do, even when faulty technique is not involved.

HOST PARASITE RELATIONS

As already noted the only positive results in transmitting Sarcosporidia to normal hosts have been with *S. muris* and *S. tenella;* these two species have each been transmitted to several foreign hosts; the methods used in transmission were by feeding either infected muscle or food contaminated by feces from infected animals; the one exception to these methods of transmission was the intra-muscular inoculation of Sarcosporidia from the opossum to the guinea pig. Failures to

transmit Sarcosporidia have been numerous. The writer in 1915 and later, has repeatedly failed to infect lambs by feeding infected sheep muscle or Balbiania. In 1927 he failed to infect the white rat, but if Arai's observations are correct, the muscle and Balbiania used may have been too old. Moulé (1888) ate raw beef containing sarcocysts without noticeable effect; Pfeiffer (1891) notes the lack of Sarcosporidia in butchers' dogs though given quantities of esophagus containing Balbiania, and numerous other failures to transmit the parasites by feeding or by inoculation have been reported. Scott (1920a, b) found that infection occurred in lambs confined with their ewes without any secondary host being present. Any theory of transmission that involves the presence of an intermediate host would seem to be excluded in the case of *S. tenella.* Scott and O'Roke (1920) after raising lambs under variously controlled conditions, found that restricted range influenced the degree of infestation more than any other factor. Since restricted range and close confinement tend to favor contamination of food, their results are easily understood. At present the theory that conforms best to all known facts is the theory that their is a resistant stage of the parasite in the feces of the host that by reason of food contamination conveys the infection to the intestine and ultimately to the muscles of a new host. The finding of spores by Arai in the blood of all mice with ripe sarcocysts suggests that their presence in the blood probably represents a step in the life history of the spore on its way from the muscle to a resistant stage in the feces. The finding of spores in the blood of old ewes by the writer adds further weight to this suggestion.

The pathological relations of Sarcosporidia to their hosts will be considered briefly under general effects attributed to Sarcosporidia, the diseases attributed to them, the pathological effects on the muscle tissue, the effects of injecting extracts or feeding the parasites. Numerous early writers, von Leiserung (1866), Damann (1867), Niederhäusen (1873), Pütz (1887), and others, have attributed serious illness and death of domestic animals to the presence of large or numerous sarcocysts. Virchow (1866) suspected that symptoms of lameness, feebleness and intermittent paralysis in swine was due to Sarcosporidia, and Wasielewski (1896) ascribed paralysis of the hind quarters and some other symptoms to the same cause. Smith (1901) observed that mice heavily infected with Sarcosporidia appeared ill and slow in their movements; Koch (1902) noticed that such mice had a puffy, bloated appearance and soon died. According to Mason (1910), heavily infected camels, in the absence of any wasting disease, were extremely emaciated and showed inflammation of the heart and its coverings with small hemorrhages and gelatinous degeneration; there was also disintegration of the voluntary muscle fibers, but no inflammatory reaction when the cysts were intact. In a two year old ox which before death showed lameness,

lowered skin reflex, short, sharp breathing and no appetite, Schneider (1917) found nothing abnormal in the internal organs; the musculature for the most part was softened, watery, flecked with yellow-greenish color, and gave the appearance of being covered with clear gray, pointed or elongated stippling; numerous Sarcosporidia were present. On the other hand, Railliet (1886) found sarcocysts in both fat and thin animals, and noted no serious results. Edelmann (1908) regarded Sarcosporidia as harmless, and Darling (1915) considered them of little pathological significance and no serious economic importance. Viljoen (1918) believes they are harmless parasites in cattle, and that they overrun the animal only when it is debilitated through other causes. Guiart (1922) is of the opinion that, except for *S. muris,* the pathogenicity is proportional to the number of Sarcosporidia present. Fantham and Porter (1914) have a similar view. Croveri (1920) thinks that ordinarily Sarcosporidia cause no serious harm, but if deficient nutrition, or disease is present, they may produce serious results and even death. There is thus a wide difference of opinion as to the general pathological effects produced by these parasites. In this connection it might be mentioned that sarcosporidiosis has been associated by Beale (1878) with cattle plague, by Watson (1909) with loco disease of horses, by Alexander (1913) and McGowan (1914, 1923) with a disease of sheep called scrapie. There is no genuine proof to indicate that these diseases are caused by Sarcosporidia. Walker (1918) finds that the symptoms characteristic of scrapie are not found in sheep with Sarcosporidia; he further notes that poor condition is frequently associated with worm infection, and he found no symptoms of "Lamziekte" in either horses or sheep that were infested with Sarcosporidia.

Numerous observations have been made with reference to the effects of Sarcosporidia on the muscle tissue. Zurn (1872) noted a change in gross appearance. Morot and Railliet (1886) observed that the flesh of sheep was watery when many sarcocysts were present. Ostertag (1905) describes cases of sarcosporidiosis in cattle associated with lameness, and noted post-mortem muscular changes associated with large numbers of sarcocysts; in one case there was paleness, loss of striations, and granular disintegration of muscle fibers. Navez (1919) concludes that Sarcosporidia, exceptionally but undoubtedly, produce inflammatory lesions; that nodules of inflammation are surrounded by an interstitial myocystis accompanied with remarkable eosinophilia. Hadwen (1922) noted that the flesh of reindeer heavily infested with Sarcosporidia was so changed that it could not have been passed as food. Creech (1922) describes "soft" or "mushy" hams as always associated with an abundance of Sarcosporidia, and he believes the lesions may be due to excessive numbers or to the toxic products eliminated from the parasites. Beller (1924) after a careful study of the effects of sarcosporidiosis on

the muscle in domestic animals, concluded that a disease of the skeletal muscles due to Sarcosporidia is not infrequently found; that it is characterized by a perimysitis in which eosinophile leucocytes have an important share; the muscle fibers react in the sense of a simple atrophy in connection with an obliterating endarteritis of the regional blood vessels; as part compensation for the destruction of contractile substance the elastic tissue in the perimysium is hypertrophied. The inflammation occurs at the time of the infiltration of the parasites while its healing in the muscle fibers runs a reactionless course. Chiwy and Colback (1926) studied the sarcosporidiosis of cattle in the Belgian Congo; clinical symptoms in infested animals are not often found; if the parasites are numerous, muscle movements show harmful effects such as lameness and myocarditis, and even endocarditis may be produced. The authors believe that sarcosporidiosis of the heart may be the cause of death in many cases which often occur where the animals must travel over long stretches of marshy country to get from one grazing ground to another. From some of this recent work is obtained important information in regard to the effects of Sarcosporidia on the muscles and their relation to disease. The writer has observed in sheep numerous lesions and pathological effects produced by *S. tenella*.

Some pathological effects have been produced by the injection of extracts of sarcocysts. Kasparek (1895) verified the work of Pfeiffer (1890), and showed that subcutaneous injections of emulsions of *S. tenella* killed mice and guinea pigs. Laveran and Mesnil (1899) prepared filtered water and glycerine extracts of both fresh and dried material; both kinds of extracts in very small doses killed rabbits, but had no effect on rats, mice, sheep, frogs or turtles. They named the toxin "sarcocystin." These results were confirmed by Rievel and Behrens (1903) who used extracts from llamas and came to the conclusion that sarcocystin is a neurotoxin. Teichmann (1910, 1911) used dried extracts of *S. tenella;* after lethal doses were given to rabbits the poison was found localized in the central nervous system bound up with its lipoids. Naturally immune animals include the guinea pig, dog, cat, ape, marmot, hen, dove, canary, paramecium; and the rabbit may be immunized. He concludes that *S. tenella* contains a true toxin which is thermolabile, filtrable, soluble in salt solution, and is toxic for rabbits; he named it "sarcosporidiotoxin." Sabrazès and Muratet (1911) prepared a pulp deprived of all microbes, from the cysts of *S. bertrami;* a small dose, from 10 cysts, produced a morbid condition; large doses produced choleralike diarrhoea and death. Cominotti (1913) used Teichmann's methods and obtained similar results; he showed that sparrows as well as rabbits are affected by the poison, and that anaphylactic shock may be produced in guinea pigs by repeated injections. Chiwy and Colback (1926) showed that one-tenth milligram of dried

extract of *S. blanchardi* is enough to kill a rabbit. Sato (1926), working on sarcocysts from the ox, dried grown cysts in a vacuum, pulverized them, and made an emulsion which was shaken 2 to 3 hours and then centrifuged. The resulting clear fluid contained the sarcosporidiotoxin, which is destroyed by heat at 50° C. for 30 minutes, by carbon dioxide, and by acids, but is resistant to alkalis; its effect is inert when swallowed, weak when injected into the brain, weaker still when injected into the peritoneum, but very active when injected into the blood of a susceptible animal. The toxic symptoms were excitement, paralytic collapse, severe diarrhoea, and death. The pathological changes indicated that the toxin acted upon the adrenal glands, the liver, the intestinal walls, and the heart. How far these various experimental results are applicable to the ordinary life history of the parasite remains to be demonstrated. That the Sarcosporidia contain a toxic principle is further indicated by some of the results of feeding.

Nègre (1907) observed what he calls a "sarcocystic reaction" before the appearance of the parasites in the muscles of experimentally fed mice. Erdmann (1910) noted that feeding spores or even the extract of spores caused destruction of the epithelial cells; the lumen of the intestine became filled with blood, and this gave a reddish appearance to the mucous membrane and musculature. In young mice clonic cramps and lameness of extremities were symptoms produced. It is suggested by this author that this reaction is an adaptation for easier entrance of the parasites, but this suggestion is hardly tenable. Crawley (1916) observed similar results. He noted exfoliation of the epithelial cells before any extensive invasion of the parasites, and described various stages in the process which resulted in severe cases in the complete destruction of villi. He has no explanation to offer. The writer (1927) noted similar effects produced by feeding Balbiania to young rats. Soon after eating several Balbiania, the young rat ceases to take an interest in its surroundings, remains hunched up in the corner of its cage, does not appear well, and is disinclined to move about. Upon opening the body the intestine gives the appearance of inflammation in the affected regions. Smear preparations show that in the regions of toxic, cytolytic effects, the contents consists of a bloody serous exudate, spores, and various sorts of cell fragments and debris. Sections verify these results and show that within two hours after heavy doses complete destruction of the villi may take place. The jejunum is invariably empty and is practically free from cytolytic effects.

Considering all the evidence in regard to the pathogenicity of Sarcosporidia, it is probable that at times these parasites produce serious effects on their hosts. The symptoms of sarcosporidiosis, however, are still unrecognized or ill defined. Perhaps these conditions are what should be expected from a generalized infection, and that a better

knowledge of their pathological effects will be obtained only when a more complete knowledge of their life history and a means of diagnosis are available. It might be mentioned here that Mason (1910) treated sarcosporidiosis in camels with sodium arsenate, and that it had no effect on the cysts. Brumpt (1927) states that it is probable that Sarcosporidia have a very feeble parasitic specificity, and cites some of the instances of transmission from one host to another that have been mentioned in this paper. However, when Darling (1910) infected the guinea pig with *S. muris* he found the parasite did not appear in the muscle until 152 days after the most favorable feeding, and the size of the parasite was reduced. Betegh and Dorcich (1912) obtained similar results after infecting a hen and two ducks with *S. tenella,* and they thought this due to the more unfavorable conditions found in birds. However, some birds, for example wild ducks, may be heavily infested with Sarcosporidia. Since *S. tenella* may develop in the mouse, guinea pig, hen and duck, with the difference in environment producing a difference in the form of the parasite, Betegh and Dorcich raise the question, what is a species among sarcocysts? One also might argue that there is some evidence here for a partial host specificity. Darling (1915) states that there are apparently two types of Sarcocysts, those with large spores, such as rat, sheep, cattle, horse and those with small spores, such as man, sloth and opossum; considering the transmission experiments mentioned, he thinks the size of the parasite may depend upon the host, irrespective of the variety of spore inoculated. However, it may be that some of the failures to transmit Sarcosporidia were due to a sort of host specificity rather than to other causes.

It has been the aim of this paper to review some of the most important work that has been done on the Sarcosporidia, to mention some of the methods of procedure that have been used in their study, and to call attention to some of the problems that are still unsolved. The student of Sarcosporidia will have no difficulty in finding new problems. However, it is believed that our knowledge of the group has advanced to the point where it is possible with some assurance outline the general limits along which future investigations are most likely to produce satisfactory results. In all likelihood the spores in the blood of the host escape to the outside by way of some of the organs of excretion, and reach new hosts by way of the alimentary canal; but what is their fate while outside of the host, and what is their fate between the time of ingestion and the time they become established in the muscle fiber several weeks later is very uncertain or entirely unknown.

Teichmann (1912) gives a bibliography that is practically complete to that date. There is listed below some of the more important references since the date of his paper.

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CONTROL OF EXTERNAL FLUKE PARASITES ON FISH

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Fluke parasites of the genera Gyrodactylus and Ancyrocephalus have been known in this country for many years and epidemics resulting in serious losses have been experienced at many fish hatcheries. The author recently found members of the genus Dactylogyrus on goldfish from Maryland, Indiana, Iowa and New York. Members of the genus Ancyrocephalus were also found on sunfish, perch, and small mouth and large mouth black bass, both in Indiana and in central New York. The economic importance of Gyrodactylus parasites has been well demonstrated and members of the genus Dactylogyrus have been reported as being very destructive in certain parts of Europe. These latter parasites have already caused serious losses among goldfish in this country and experiments are under way which indicate that members of the genus Ancyrocephalus, and to a certain extent those of Dactylogyrus, are destructive to certain native nest building fishes, not only in hatcheries but also in open ponds and streams.

Numerous methods have been devised for killing external parasitic flukes, but no method, so far as the author can learn, claims complete destruction of the parasites, and no method has thus far been worked out for pond treatment of infected fish.

The author has found methods which have proven especially successful when used on a large scale commercially. These experiments were carried out at a large fish hatchery, and in experimental ponds on the campus of Hamilton College. Sunfish and goldfish were used for the experimental work which is recorded here, but it is probable that the methods as worked out can be used for most fresh water fishes. There is, however, a marked difference in the toleration by different species of fish to treatment with chemicals. The age, size and species of fish are factors which must be considered, and experimental tests should always be made before large numbers of any species of fish are placed in chemical solutions for treatment of parasitic infections.

Approximately twenty different chemicals, many of which have been recommended at one time or another as "dips" for parasitized fish were used. At first it looked as if the acetic acid treatment as recommended by Embody (1924) would prove most effective, but further experimentation showed that potassium permanganate was much superior. It was found that acetic acid, used as a "dip" at the strength of 0.2% for two minutes, failed to kill a good many of the Dactylogyrus and Ancyrocephalus flukes which were more or less imbedded in the

mucous of the fishes' gills, and also many of the genus Gyrodactylus which were still inside of their mothers but almost ready to be born. It seems probable that these two criticisms can be applied to all of the "dip" methods. Hence it appeared that better results would be obtained if one used a weaker solution of some chemical, and prolonged the treatment for an hour or more. With this idea in mind all of the chemicals referred to above were tried, but since potassium permanganate proved so much more satisfactory than any of the others, discussion of control methods will be restricted to experiments with this chemical.

LETHAL DOSAGE OF POTASSIUM PERMANGANATE

In order to ascertain the possible difference between the lethal dosage of potassium permanganate for the fish and for the parasites, goldfish laden with both Gyrodactylus and Dactylogyrus flukes were placed in different dilutions of potassium permanganate for twenty-four hours. In each of eight aquaria containing varying dilutions of potassium permanganate six fish about two inches long were placed. The dilution in aquarium I was in the proportion of 1 gram of potassium permanganate to 100 liters of water; in aquarium II, 1 gram to 200 liters; in aquarium III, 1 gram to 300 liters; in aquarium IV, 1 gram to 400 liters; in aquarium V, 1 gram to 500 liters; in aquarium VI, 1 gram to 600 liters; in aquarium VII, 1 gram to 700 liters, and in aquarium VIII, 1 gram to 800 liters. Similarly six fish four inches long were placed in each of another series of aquaria, and also six fish six inches long were placed in each aquarium of a third series.

In the first series the fish and flukes in aquarium I were dead in one hour. In aquarium II the fish and flukes were dead in two hours. In aquarium III, IV and V the fish were all alive at the end of the 24 hour period but the flukes were all dead. In aquarium VI the fish were all alive and some of the Dactylogyrus flukes were also alive but none of the genus Gyrodactylus. In aquarium VII all fish and many flukes were alive, while in aquarium VIII all fish and flukes survived. The results obtained in the second and third series were very similar to those in the first, except that the fish, being larger, were able to withstand longer the more concentrated solutions.

The above results show that a solution of potassium permanganate as dilute as 1 gram to 500 liters of water (1 pound to 59,912 gallons) proved to be lethal for flukes, but it required approximately 1 gram of the chemical to 200 liters of water (1 pound to 23,965 gallons) to kill the fish. It was also shown that it required a slightly greater concentration of the permanganate to kill flukes of the genus Dactylogyrus than those of the genus Gyrodactylus.

As a result of the above experiments it was decided to use potassium permanganate at the rate of 1 pound to 32,000 gallons of water as the basis for further experimental work.

In order to determine the length of time necessary to kill the flukes in this strength of potassium permanganate (1 pound to 32,000 gallons of water) another experiment was undertaken. Goldfish of different sizes that were parasitized with both Gyrodactylus and Dactlogyrus flukes were placed in aquaria containing the above dilution of potassium permanganate and left for different intervals of time. At the end of one-half hour all flukes were apparently alive. Likewise at the end of three-quarters of an hour all flukes were alive but decidedly weakened as they moved very little. At the end of 1 hour all Gyrodactylus flukes were dead but some of the Dactylogyrus flukes were still alive. At the end of one and one-quarter hours only an occasional fluke was found (Dactylogyrus). At the end of one and one-half hours all flukes were dead. In order to be sure that the flukes would not revive some fish that had been in the chemical for one and one-half hours were placed in fresh water aquaria and examined 24 hours later but in no case were living flukes found on fish that had been in the above dilution of potassium permanganate for one and one-half hours or longer. Since the chemical, when diluted as used in this experiment, is not very toxic to fish and apparently causes no permanent injury, as will be pointed out later, it is recommended that fish be allowed to remain in the solution for two hours where complete destruction of the external fluke parasites is desired.

THE TWO HOUR METHOD

Immerse fish in a solution composed of 1 pound of potassium permanganate to 32,000 gallons of clean water for two hours. The tank should be thoroughly washed before being filled for use. Either cement or steel tanks are suitable for this purpose but wood should be avoided as it hastens the oxidation of the chemical. Up to date over 500,000 mature goldfish have been treated by this method without any fatalities, and though many of them were examined afterward, no parasites were found. On one occasion 1,200 prize breeders, averaging about 1 foot in length, were treated as described above. Several had been examined previously and had been found to be heavily infested. Twenty-four hours following the treatment one of them was killed and though the gills were carefully examined with a binocular microscope, no parasites could be found.

The advantage of this prolonged treatment in a comparatively weak solution of potassium permanganate is that it kills all of the fluke parasites and there is little if any perceptible immediate or subsequent injury to the fish resulting from exposure to the chemical. The above method makes it possible for breeders of fish to rid a hatchery entirely of fluke parasites. It also makes it possible for owners of hatcheries to ship fish that are free of flukes.

THE DIP METHOD

The immersion of fish from one and one-half to two minutes in a solution of potassium permanganate composed of one ounce of the chemical to 15 gallons of water (1 pound to 240 gallons) will kill most of the flukes. I had better results with potassium permanganate than with acetic acid as a dip and it injured the fish less. During my experiments a few of the fish died after being immersed in the solution for two and one-half minutes, but none were killed by an immersion for two minutes.

THE POND METHOD

Any chemical to be successful for the treatment of parasitized fish in open ponds must be one that will be effective for a comparatively short time, after which it will oxidize or in some other way become ineffective. Ponds differ greatly in many ways. The deeper the pond and the more organic matter it contains, the more difficult it is to use the potassium permanganate treatment effectively. Best results were obtained in ponds that were less than three feet in depth. If enough organic matter is present to hasten oxidation materially, it is often necessary to use a stronger solution of the chemical. Most fluke epidemics occur among young fish that are about one inch in length. Successful treatment of fish of this size even in aquaria, has been considered impossible. Yet I was able successfully to stop fluke epidemics on fish of this size in open ponds.

In experimental work I varied the strength of the solution from one pound of the chemical to 25,000 gallons of water, to one pound to 40,000 gallons. Only with the strongest solutions used was there the least evidence of injury to the fish. The general procedure for the method worked out for use on a large commercial basis was as follows: The water was shut off so that it would not flow through the pond, the capacity of the pond was calculated in gallons, the amount of the chemical to be used was ascertained, dissolved in large steel tanks and sprayed over the surface of the pond by means of a gasoline force pump. In every instance care was exercised to cover the surface as evenly as possible. The usual strength used was one pound of the permanganate to 30,000 gallons of water. In case of young fish about an inch in length one pound was used to 32,000 gallons of water. Where ponds contained a large amount of organic matter the dosage was increased somewhat but never more than that required to make the dilution equivalent to one pound to 25,000 gallons for older fish, and 30,000 gallons for very young fish.

Parasitized fish naturally seek the surface of ponds for oxygen especially when the gills are infested, and hence tend to come in contact with the more concentrated solution before it completely diffuses through the water of the pond. In open ponds permanganate becomes ineffective,

due to oxidation, in about two hours. Complete destruction of fluke parasites is not claimed for this method. It has been found by use on approximately one hundred ponds containing all the way from 25,000 to 300,000 gallons of water, that practically all of the younger parasites are killed and a large percentage of the older ones. In every case where used it has broken the epidemic and permitted the fish to get ahead of the parasites. In a few cases evidence of reappearance of the epidemic occurred in about 7 to 10 days following the use of the permanganate, and when this happened a second dose of the chemical was used.

KILLING FLUKES AND THEIR EGGS LEFT IN PONDS WHEN FISH ARE REMOVED

Heavy losses are frequently experienced at hatcheries when mature fish which are infected with flukes are taken from ponds and young fry are introduced as soon as the ponds have been refilled with water. Mature fish of many species are often able to carry external flukes without showing evidence of the infection, yet the new stock of young fry, when put into the ponds, frequently die in great numbers. In the development of most species of Dactylogyrus the egg stage requires about four days and the larval stage five days during the warm summer months, while during the early spring (March and April) the combined egg-larval period may last for eighteen days or longer, depending on the temperature of the water. Even when ponds are empty, the moist mud frequently holds enough water to keep these organisms alive, especially the egg and larval stages.

Experimental tests showed that if hydrated lime is sprinkled on the bottom of ponds so as to cover the surface of the mud or gravel as soon as they are thoroughly drained and the fish are removed, both eggs and living flukes will be killed inside of one hour. The above treatment of ponds containing different stages of external flukes makes possible the refilling and almost immediate restocking with young fry. The experimental work was verified by excellent results in pond treatment. Over sixty ponds were limed and no epidemics developed after the ponds were restocked. Due to lack of thoroughness in applying the lime in some of the ponds, flukes appeared on some of the fish but not in sufficient numbers to cause losses.

Where ponds are left without fish for a period of time in order to rid them of flukes it is necessary in case the fish were parasitized by Dactylogyrus flukes to keep the ponds free of fish for about nine days during warm summer months, or for three to four weeks early in the spring. Some authors have claimed that two or three days is sufficient but this is not correct. In the case of Gyrodactylus infection, where there are no egg and larval stages, the parasites usually die if

the ponds are left without fish for two or three days. This proved to be true during June and July but it was not possible to make tests earlier in the spring.

ACQUIRED RESISTANCE IN TYPICAL DACTYLOGYRUS INFECTION

Great variation was observed in the size and numbers of Dactylogyrus parasites on fishes. In all cases observed the largest flukes were found on fish which had more recently become parasitized and this was especially noticeable in the case of young fish. Fish that had been parasitized for a year or more, unless in poor physical condition, harbored very few flukes and those that were found were always much smaller than those that were found on fish that had recently become parasitized. This seems to indicate that the fish develops a partial or complete immunity to the parasites after a time. In the lappets near the anterior end of the fluke are ducts which drain glands that lie near the pharynx. Can it be possible that this glandular substance is absorbed by the fish and causes a substance to be produced in its body which is unfavorable to the fluke?

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A NEW FLAGELLATE FROM THE INTESTINE OF *LUCILIA CAESAR* L.*

R. W. Glaser

In July, 1929, six out of twenty, or 30 per cent, wild adults of the green-bottle fly, *Lucilia caesar* L., were found parasitized with a protozoan having two flagella. The flagellate occurred in large numbers within the intestine of the flies. Examinations of *Lucilia* flies in August and September did not again reveal the microörganism. Since much has been written lately about the protozoa of insects, especially concerning Herpetomonads and their relation to the Leishmanias, it was considered appropriate to describe the species. The author has recently succeeded in obtaining bacteria-free cultures of the microörganism. The technique and media employed during this work will be described elsewhere. The characters given here are those when the flagellate is cultivated after an incubation of from 18 to 82 hours at room temperature (22 to 29°C.). Characters described from cultures checked with the characters of the parasitic forms found within the intestine of the flies.

The protozoan corresponds slightly with another biflagellated form *Lucilia* sp. (*jedensis?*) described but unclassified by Yamasaki (1927). This species, however, differs from Yamasaki's morphologically and the measurements given by this author do not agree with those of the form here recorded. This species appears to be new, but since its exact position is not sure I have at present refrained from naming the microörganism. However, it appears naturally to belong to the family Monadidae among the Mastigophora.

DESCRIPTION OF PARASITE

Elongated or oblong football-shaped protozoan. In some cultures anterior end is often pointed whereas posterior end is rounded. In other cultures posterior often ends in a sharp point while anterior is rounded. Spherical forms are common. Reproduction occurs by division into two. Delicate periplast surrounds each cell. Two flagella of nearly equal length protrude from anterior end. Nucleus occurs nearer anterior than posterior part of body. Two blepharoplasts exist. A deeply staining parabasal body exists usually along or near periphery at or to one side of anterior end. Blepharoplasts are intimately associ-

* From the Department of Animal Pathology of the Rockefeller Institute for Medical Research, Princeton, N. J.

ated with parabasal body and are difficult to detect. Rhizoplasts not seen, probably due to their delicacy and to close proximity of blepharoplasts to parabasal body. Two or three digestive vacuoles often seen, and in contaminated cultures contain bacteria. No undulating membrane nor cystostome present. Cysts were never seen, not even in old or unfavorable cultures. The motility of protozoan is characteristic. Progresses in an intermittent manner due to whip-like snapping of flagella. Preparations were studied by fixing in hot Schaudinn's fluid and staining with 0.5 per cent water-soluble iron hematoxylin, counterstaining with 0.5 per cent water-soluble phloxine. Great care must be exercised in dehydrating and in clearing.

Correlation Table

Length in μ of Flagellate	Breadth in μ of Flagellate										
	3.2	3.6	4.0	4.4	4.8	5.2	5.6	6.0	6.4	6.8	7.2	Total
5.6	..	..	1	..	..	..	..	..	..	..	..	1
6.0	..	..	..	..	1	..	..	..	..	..	..	1
6.4	..	..	1	1	1	..	..	..	..	..	..	3
6.8	..	..	..	..	..	..	..	..	..	..	..	..
7.2	..	..	..	..	..	..	..	..	..	..	..	..
7.6	..	..	..	..	..	1	..	..	..	..	..	1
8.0	2	2	1	1	5	1	..	..	1	..	1	14
8.4	1	..	..	..	..	..	..	..	..	..	..	1
8.8	..	..	..	..	..	..	..	..	..	..	..	..
9.2	1	..	..	..	..	..	..	..	..	..	..	1
9.6	..	..	..	..	1	..	..	..	..	..	1	2
10.0	..	..	..	..	..	..	..	..	..	..	..	..
10.4	..	..	..	..	..	..	..	..	..	..	..	..
10.8	..	..	..	..	..	..	..	..	..	..	..	..
11.2	..	..	..	..	1	..	..	..	..	..	..	1
Total	4	2	3	2	9	2	..	..	1	..	2	25

The accompanying table represents the correlation between length and breadth of the flagellate. Twenty-five measurements were made for each character. The finer divisions on the table are based on multiples of 1.6μ which represents the value of the finest division on the ocular scale used.

It will be seen that the length range of the flagellate equals 5.6 to 11.2μ and that the breadth range equals 3.2 to 7.2μ. The frequency distribution gave 8.0μ for the modal length and 4.8μ for the modal breadth. The mean length is 7.76μ and the mean breadth 4.6μ.

The following data are also based on 25 measurements each. Diameter range of spherical forms equals 3 to 8μ with a mean of 5.79μ. Distance from middle of nucleus to posterior end of cell ranges from 3.2 to 5.6μ with a mean of 3.9μ. Distance from middle of nucleus to edge of parabasal body is practically constant and equals 1.6μ. As mentioned, the parabasal body is usually at the extreme anterior end. We have found thirteen cases, however, in which the body was at some

distance from the anterior. For these cases the range of distance from parabasal body to anterior end equals 1.6 to 4.0μ with a mean of 2.5μ. Data on flagellar lengths are only approximate due to many curves which make accurate measurement difficult. Approximate range in flagellar lengths equals 10 to 21μ with a mean length of 14μ.

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NOTES ON THE LIFE HISTORY OF *HAPLOBOTHRIUM GLOBULIFORME* COOPER, A TAPEWORM OF *AMIA CALVA* L.*

LYELL J. THOMAS

Haplobothrium globuliforme was first described by Cooper (1914, 1914a) and was indicated by him as having a small scolex which "can scarcely be distinguished from the first joints." Later (1917) he discovered his error—that the true scolex "has as organs of attachment four eversible proboscides instead of two simple modified bothria." This structure in many ways resembled the scolex of tapeworms belonging to the order Trypanorhyncha but because of the reproductive system he retained it within the order Pseudophyllidea. This same author (1914a) noted the complete development of coracidia in eggs in the uterine sac and well describes their erratic swimming movements when they hatched out in saline solution. Later in 1918 he alludes to "the original larva which resembles the bothriocephalid plerocercoid, excepting for the peculiar scolex" which he found in the intestine of the final host. Other than this nothing was known concerning its life history.

During the summer on August 5, 1928, at the University of Michigan Biological Station, the present author found coracidia hatching out in the wash water used in the examination of the intestinal contents of an *Amia calva* taken from Douglas Lake, Michigan. The hatching took place only a few minutes after the eggs were in water (Figs. 1 and 2). A tow of crustacea was taken from Douglas Lake and unhatched eggs and coracidia were added to ten two inch stender dishes. Cyclops were noted taking the coracidia. One coracidium was observed to slough off the ciliated embryophore (Fig. 3) in Ringer's solution modified for fish so others were placed in this same solution with the result that the ciliated embryophores were shed and the embryos sank to the bottom of the dish. Here within the remaining mantles the hexacanth embryos continued their active movements for nearly a week, whereas those left in lake water survived only a day. On August 8, *Cyclops brevispinosus* was found with four procercoids in early stages of development within the body cavity. These measured about 75 by 130μ as compared with the onchospheres which measured from 30 to 42μ in length. Thus in about three days their size was

* Contribution from the University of Michigan Biological Station and the Zoological Laboratory of the University of Illinois, No. 368.

increased about four times. The examination of another *C. brevispinosus* showed similar procercoids.

An examination of ten Cyclops on August 13, picked at random from dishes, showed four infested with procercoids which measured about 217μ when fully extended (Fig. 4). One (Fig. 5) out of five procercoids dissected out of the body cavity of a Cyclops was without a cercomer. This retardation of the development of some of the procercoids in heavily infected individuals has been noted by the writer before in other species, also by Essex (1927:45). It is possibly due to lack of food materials for so many individuals. The end opposite the cercomer in these fully developed individuals was stretched out from time to time as an extremely active proboscis. A short distance back of the proboscis-like end in some specimens, a clear area was visible and traces of the excretory canals were seen near the cercomer. A few minute and scattering refractive bodies were noticed throughout the main body.

The end of the summer session at the Biological Station brought further experiments to a close. Again in the summer of 1929 additional data were added to those of the previous summer. A trammel net was set in Burt Lake, Michigan, at the mouth of Maple River, in hopes of catching infested Amia. During the course of the summer seven bowfins were caught. From a fish company in Cheboygan, Michigan, an adult female Amia was secured that had been taken at Nobenway, Lake Michigan, July 1, 1929. One immature adult *H. globuliforme* with a complete scolex was found as well as twelve mature *Proteocephalus perplexus,* together with numerous immature proteocephalids. By carefully scraping the intestinal mucosa in normal salt solution ten immature bothriocephalids (Figs. 6, 7 and 8) were found. The scolex of one of these young bothriocephalids (Fig. 9) pressed out under a coverslip showed an intricate excretory system (Fig. 9a), the tubules of which after anastomosing in the anterior part continued posteriorily as three separate trunks. This was strikingly similar to the excretory system of *H. globuliforme.* The writer also noted marked similarity in the musculature and the presence of minute striations on the scolex that are the same in Haplobothrium. These same striations are present in the procercoid. A few also contained a scattering of minute nodules that are present in this same tapeworm over the surface of the scolex distally. An attempt was made to find indications of beginning pouches for the proboscides that are present in the adult *H. globuliforme,* but this has not been demonstrated to the satisfaction of the writer. The ten young bothriocephalids were killed in Mayer's picrosulphuric killing fluid and measured in length fully extended as follows:

0.395, 0.713, 0.929, 1.092, 1.47, 1.5, 2.5, 2.7 and 3.01 mm. The longest one had indications of internal segmentation to form ten proglottids. These may not prove to be larval stages of *H. globuliforme* but feeding experiments now in progress are hoped to clear up this point.

On July 14, 1929, an adult *H. globuliforme* was found in the stomach of *Amia calva* from Burt Lake, Michigan, together with numerous proteocephalids. Eggs streamed out of the posterior proglottids when the worm was placed in cold well water.

The first eggs out hatched in a few minutes, those given off later required several hours. Cyclops from a laboratory culture were added in six fingerbowls with about a half inch of water. When examined the next day *Cyclops brevispinosus* and *C. viridis* var. *americana* were found infested by dissecting out the procercoids from the body cavity. Four days later larger and more active procercoids were present. This same lot was examined again July 24 and numerous procercoids with fully formed cercomers were dissected out in a modified Ringer's solution (Fig. 10). Their movements were leech-like, the cercomer and the main body being contracted and extended to considerable lengths. One procercoid (Fig. 11) shed its cercomer in the Ringer's solution. Traces of the excretory system were seen and the elongated proboscis-like end was slightly bulged about 12μ back from the tip. Minute transverse striations were present over the entire cortex. Some of these larvae were killed by means of two per cent osmic acid and mounted unstained in euparol. In this condition the cercomer fully extended measured 54 by 22μ and the body part fully extended 150μ long and 30μ at the widest part. At this stage of development no structures were evident that would indicate the future four protrusible proboscides of the adult worm. In general the procercoids resemble those of other Pseudophyllidea.

Another adult tapeworm with mature proglottids was taken July 22 and Cyclops were added to the hatching eggs, *C. viridis* var *americana* dissected out July 23 had hexacanth embryos in the intestinal wall (Fig. 12) and body cavity.

Fifty young suckers taken from the same locality at the mouth of Maple River were examined as possible second intermediate hosts but no bothriocephalid plerocercoids were found. An examination of the stomach contents of all dogfish taken indicated crayfish as a considerable part of their diet. Forbes and Richardson (1920) in their findings from April to September include minnows though crayfish constituted forty per cent of the diet of those examined. Dr. Alvin R. Cahn kindly loaned the writer his collections of parasites taken from *Amia calva* from Oconomowoc Lake and Oconomowoc River, Wisconsin. Mature

H. globuliforme were present in fish taken June 17, 1924, and August 30, 1925. Dr. David Thompson of the Illinois State Natural History Survey obtained two bowfins for the author from Chillicothe, Ill., which were examined December 7, 1929. Two immature tapes were taken from one and one from the other. A series of sketches was made of the movements of the scolex of one of these young tapes in Ringer's solution (Figs. 15-18) which showed great contracting power and diversity of form. In one stage of contraction (Fig. 18) the four proboscides appeared as in a clear bladder-like structure almost detached from the rest of the scolex. Data from the collection dates would seem to indicate that infections of *Amia calva* may not be confined to one season but may be continuous. This is under investigation at the present time.* The determination of the exact manner whereby the proboscides are developed is as yet unsolved and will be of considerable taxinomic interest for especially among certain European workers, Southwell (1925), the type of scolex has been considered of utmost importance in the determination of Cestode orders. Nybelin (1922) considers *Haplobothrium globuliforme* a doubtful Pseudophyllidean. Poche (1925: 358-61) places this worm under the order Tetrarhynchidae, as a suborder Haplobothriinea and on page 360 says: "Und zwar betrachte ich Haplobothrium als die primitivste uns bekannte Form der Tetrarhynchidea." Woodland (1927: 535) in a foot note questions Poche's conclusion that it is a primitive Tetrarhynchid and says it is more likely a case of parallel development. He also on page 537 considers that the Pseudophyllidea may have given rise to the Tetraphyllidea. However, only by carefully controlled feeding experiments does the writer hope to establish the identity of these young bothriocephalids from *Amia calva* and to clear up some taxinomic difficulties involved in the development of the proboscides.

Addendum. A slide containing two cestode cysts mounted in balsam was sent to the writer by Prof. Stephen R. Williams, Miami University, February 24, 1930. He stated that the worms were taken from the liver of a sunfish, the summer of 1916 at Cedar Point, Ohio. The writer has identified them as *H. globuliforme* described by Essex (1929) and one measures as follows: bladder-like structure 1.26 by 0.6 mm., neck 0.88 by 0.33 mm., four proboscides extended each 0.35 mm.

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* After this work had been accepted for publication the writer's attention was called to a publication by Essex, H. E., 1929: The life-cycle of *Haplobothrium globuliforme* Cooper 1914, Science **69**:677-678. Essex found five out of eight *Ameiurus nebulosus* taken in the Mississippi River near Homer, Minn., with larval cestodes encysted in the liver. These young tapeworms had scolices closely resembling that of *H. globuliforme*. These may prove to be the plerocercoids of *H. globuliforme* and if so, complete this most interesting life cycle.

SUMMARY

1. The eggs of *Haplobothrium globuliforme* Cooper 1914 mature in the uterine sac and hatch out coracidia shortly after being laid.

2. *Cyclops viridis* var. *brevispinosus* and var. *americana* act as intermediate hosts.

3. The infective stage of the procercoid develops in about ten days in the body cavity of the Cyclops as a typical Pseudophyllidean.

4. The proboscides of the adult worm are secondary structures formed later in the life cycle.

5. Young bothriocephalids found in the mucosa of the Amia are suggested as possible stages of *H. globuliforme*.

6. Plerocercoid stages occur in sunfish.

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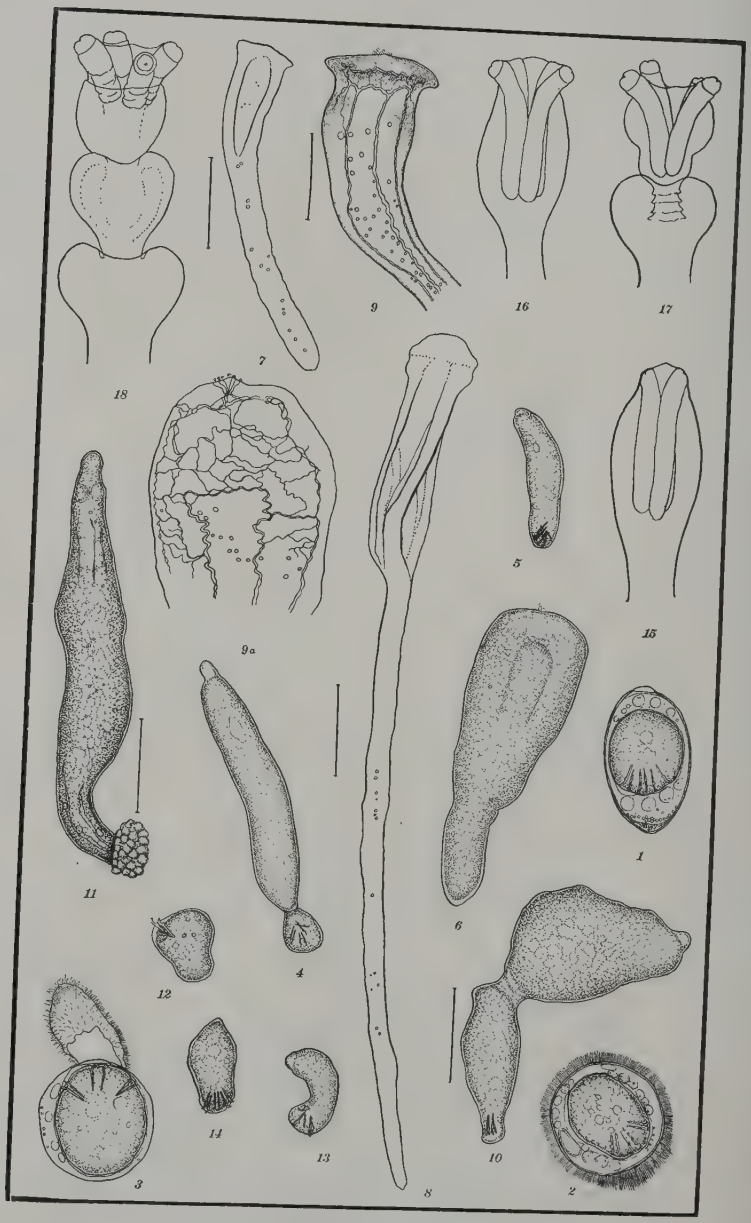

PLATE XI

Explanation of Plate XI

Camera lucida drawings except figures 15, 16, 17, and 18; scale, 0.20 mm. for figures 7, 8, 9, and 9a and 0.05 mm. for figures 10, 11, and 12.

Fig. 1.—Egg from uterine sac containing the ciliated embryo.

Fig. 2.—Coracidium just hatched.

Fig. 3.—Coracidium sloughing off ciliated embryophore in modified Ringer's solution.

Fig. 4.—Procercoid from body cavity of Cyclops, infective stage.

Fig. 5.—Procercoid from same lot as Fig. 4, cercomer formation retarded.

Figs. 6, 7, 8.—Young bothriocephalids from intestinal mucosa of *Amia calva*. L.

Fig. 9, 9a.—Same as 8, showing details of excretory system in scolex, musculature, etc., also bubble-like secretion at apex of scolex which appeared when placed in Ringer's solution.

Fig. 10.—Infective procercoid from body cavity of Cyclops showing extension of cercomer and contraction of body.

Fig. 11.—Infective procercoid which shed its cercomer in a Ringer's solution modified for fish.

Fig. 12.—Hexacanth embryo from the intestinal wall of the Cyclops the day following the infection.

Figs. 13, 14.—Developmental stages from the body cavity of the Cyclops.

Figs. 15-18.—Freehand sketches of the adult scolex at intervals after the worm was placed in modified Ringer's solution.

COMMUNITY SURVEYS FOR *ENDAMOEBA HISTOLYTICA* AND OTHER INTESTINAL PROTOZOA IN TENNESSEE; FIRST REPORT.*

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The studies to be reported represent a cooperative project of the Department of Preventive Medicine of Vanderbilt University with the Tennessee State Department of Public Health. The routine survey examinations for intestinal protozoa were made on specimens collected in connection with a general survey of the state for intestinal parasites. In the helminth studies the Department of Helminthology of the Johns Hopkins School of Hygiene was also a cooperating agency. The survey began in December, 1928, and is still under way, but the results to be reported are those recorded between July 1 and December 3, 1929.

The specimens so far examined have come from white schools and communities in the rural sections of East and Middle Tennessee. Many of the subjects live in primitive conditions in the hills of the Cumberland Plateau. Others are average farm groups, and still others are from towns of one to two thousand inhabitants. The large majority of the specimens have been collected through schools, so that the school age group of 6 to 17 years greatly predominates. A considerable number of preschool age children have also been examined, and fewer adults. A small number of studies have been made on complete families. The distinctive feature of the survey is that the subjects were all living in their homes, that is, in their normal environment. So far as can be determined, this is the first large survey for intestinal protozoa in which the subjects were not in an unnatural evironment, such as a hospital, asylum or military organization.

TECHNIQUE

Specimens were collected in one-ounce tins and sent by mail or messenger to the laboratory. At the time of examination specimens were from one to ten days old. The results are based, therefore, upon examination for cysts only, and *Trichomonas hominis* is eliminated entirely. The technique of preparing specimens for protozoal examination was as follows:

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* From the Department of Preventive Medicine and Public Health, Vanderbilt University, and the Tennessee State Department of Public Health.

* Read before the American Society of Parasitologists, Des Moines, Iowa, Dec. 31, 1929.

About a gram of feces was broken up by shaking in a 25 cc. test tube with about an equal quantity of sterilized sand and 10 cc. of 0.85 per cent table salt. After allowing the sand and coarser particles to settle, the suspension was filtered into a 50 cc. round-bottomed centrifuge tube through a small funnel containing a layer of thin "sheet cotton" made absorbent by previous soaking in gasoline. Normal saline was then poured through the sheet cotton up to about 40 cc. in order to wash through cysts which had been held back. The suspension of cysts and finer fecal particles was then centrifuged at 1800 revolutions for two minutes, which precipitated all the cysts but left some batceria and débris still in suspension. The supernatant fluid was poured off and a small portion of the sediment was mixed on a slide with an iodin-potassium-iodide-eosin solution, and a coverslip was applied for examination. The entire coverslip was then traversed with the 16 mm. lens of the microscope, and several parts of it with the 4 mm. lens. Any doubtful cyst and always cysts suspected of being *E. histolytica* were examined with the oil-immersion lens. A 10 × ocular was used. All specimens in which there was doubt about the identification of *E. histolytica* were stained with iron hematoxylin for confirmation.

Although the above method of preparing specimens for examination does not concentrate the cysts very much, it has two advantages; namely, that the cysts are evenly distributed through the specimen, and that there is good separation of fecal particles so as to make the cysts more easily visible. In a series of 45 persons on whom six examinations have been made and cysts of *E. histolytica* found in one or more specimens, 22, or 49 per cent, were positive on the first examination. An average of 22 were positive on each of the six examinations, showing that the first examination was of average accuracy. Previous workers (Jepps 1921, Kessel 1924) have determined that the straight smear method of examination will reveal about 44 per cent of the carriers of *E. histolytica* on the first examination. With the technique used in the present survey 50 per cent has been taken as the proportion found on the first examination, and this is used as a basis for calculating the actual incidence in the population groups studied. Since it is hoped to improve the technique so as to produce more concentration of cysts, the incidence of the other protozoa is presented at this time on a basis of one examination only.

RESULTS OF GENERAL SURVEY

Table 1 shows the results of the present survey for the cysts of the six common species of human intestinal protozoa. *E. histolytica, E. coli* and *Giardia* run higher than has been found in most previous surveys, while *E. nana* runs considerably lower. *Iodamoeba* and *Chilomastix* conform closely to the incidence in other surveys. This report will deal further only with observations on *E. histolytica.*

Table 2 gives a comparison of the incidence of *E. histolytica* in this and previous surveys on the basis of six examinations per individual. The figures for the first five surveys are taken from Kessel and Svensson (1924), and Kessell (1925). The results of Doctor E. C.

Faust's small survey in Wise County, Virginia, in 1928 have not yet been published (Faust 1930), and I am indebted to him for the use of his data. It will be noted that the incidence of *E. histolytica* in Faust's survey and in the present one are higher than in any of the others except that from Korea, which stands between these two. It would seem, therefore, that in the type of population which we have

TABLE 1.—*Incidence of Intestinal Protozoa in Tennessee*

Summary of Survey, July 1 to Dec. 3, 1929

Basis of One Examination	
Total number of specimens	4,987
Total positive for one or more protozoa	2,759
Per cent positive for one or more protozoa	55.3

Parasite	Number Positive	Per Cent Positive
Endamoeba histolytica	861	17.3
Endamoeba coli	1,578	31.7
Endolimax nana	489	9.8
Iodamoeba bütschlii	288	5.8
Chilomastix mesnili	191	3.8
Giardia lamblia	942	18.9

TABLE 2.—*Incidence of* Endamoeba histolytica *in Various Surveys*

Author	Country	Type of Population Group	Number Examined	Per Cent Positive Basis of 6 Exams.
Dobell, 1921	England	Summary of all civilian reports	3,146	7 to 10
Kofoid and Swezy, 1920	U. S. A.	Home Service Troops, never abroad	53	26.5
Boeck and Stiles, 1923	U. S. A.	Foreign and Home Troops, Hospitals, Asylums, etc.	8,029	8 to 10
Kessell and Svensson, 1924	Peking, China	Hospital and Orphanage	275	29.5
Kessell, 1925	Seoul, Korea	Hospital	153	41
Faust, 1928	Wise County, Virginia	In their homes	463	45.4
Meleney, 1929	Tennessee	In their homes	4,987	34.6

been studying, the incidence of *E. histolytica* is worthy of attention and may be an important health problem.

The variation in incidence of *E. histolytica* among different counties and smaller groups in the present survey is very great. Among the twenty-two counties studied, the incidence varies from 56 to 17 per cent. Four of the counties have an incidence of over 50 per cent. Three of these are adjoining backward counties in the upper valley of the Cumberland River; the fourth is in the moutains of East Tennessee and is without railroad, telephone or telegraph communications. Incidentally, three of these four counties have the highest incidence of Ascaris so far found in Tennesee.

Two of the high-incidence counties have been analyzed by communities, and here the variation in incidence of *E. histolytica* is even greater than in whole counties. One school had 28 positive out of 61, or 46 per cent, on one examination, which is 92 per cent on a basis of six examinations. Ten schools had an incidence of between 60 and 80 per cent on the basis of six examinations. Six schools, on the other hand, had an incidence of 20 per cent or less, 14 per cent being the lowest. It is evident, therefore, that the factors producing a high incidence are not uniformly present even within these counties of high incidence, but that they must be searched for in smaller units of population. This was done in a region in Fentress County called Clark Range, where the initial school survey had shown an incidence of 40 per cent. It was possible to study five families in this region.

DETAILED STUDY OF FIVE FAMILIES

These five Clark Range families were visited for the purpose of studying their environment and other factors which might produce a high incidence of *E. histolytica*. They lived in an undeveloped, rocky country. The economic status of each family was fairly good, although they seemed to have little source of income. An attempt was made to secure six fecal specimens from each individual, and this was possible in 23 out of the 27 persons in these families. It was found that 23, or 85 per cent, of the individuals carried *E. histolytica*, and 17, or 74 per cent, of the carriers had had attacks of bloody diarrhea. The earliest history of such an attack was in 1920, and most of them were within the last three years. Clinically some of the attacks might have been either bacillary or amebic dysentery, but a number of individuals reported several attacks during the same year, which is more suggestive of amebic than of bacillary dysentery.

Table 3 shows certain results of this survey. In the inspection of the family itself the intelligence status and personal sanitation appeared to average less than "fair." Water as a source of the infection seemed unlikely because four of the families had wells drilled through the rock strata. Flies were numerous in all of the houses except one which was screened. One house had a fly-proof privy, but every member of the family carried *E. histolytica*. No gross fecal pollution of the ground, such as is commonly found about houses in which families with heavy Ascaris infestation live, was discovered about any of these houses. Only five persons among the twenty-seven harbored Ascaris, and these infestations were all light. Six harbored hookworm, five of these being in one family.

In analyzing the data from the individual histories and stool examinations of these families, special attention was paid to the mother, who

TABLE 3.—*Environmental Study of Families, and Relation of Mothers to High Incidence of* E. histolytica

Name of Family	Number in Family	Carriers of E. histolytica	Number With History of Bloody Diarrhea	Nutritional Status of Family	Intelligence Status of Family	Personal Sanitation of Family	Water Supply (Well)	House Screened	Flies in House	Privy	Number Harboring Ascaris	Number Harboring Hookworms	Member of Family With Earliest History of Bloody Diarrhea	Mother's Stool Examinations for E. histolytica — Specimen Number					
														1	2	3	4	5	6
Wilson	5	5	5	Poor	Fair	Poor	Drilled	Yes	Few	Open back	2	3	Mother	++	+++	++++	++++	++	+
Heard	6	6	6	Good	Good	Poor	Drilled	No	Many	None	2	0	Mother	++	+	+	++	0	0
Choate	7	4	3	Fair	Poor	Poor	Drilled	No	Many	None	0	1	Mother	0	++	++	+	+	0
Norman	4	4	2	Good	Fair	Fair	Dug	No	Many	Good	1	0	Father	+++	++++	++++	++++	0	+
Norris	5	4	1	Fair	Poor	Poor	Drilled	No	Many	None	0	0	Father	++	++++	++++	+	++++	+

was the chief food handler. The data from the mothers is also given in Table 3. It will be seen that in three of the families the mother was the first member to have an attack of bloody diarrhea. In each case the first attack had occurred within the past nine years. In the other two families, although the mother denied having had bloody diarrhea, she was the heaviest carrier of *E. histolytica* as far as our examination could determine.

COMMENT

These facts suggest very strongly that in families whose members possess a low degree of intelligence and practice bad personal sanitation, and in which several members are carriers of *E. histolytica,* the source of spread of the infection is often the mother. She prepares the food, feeds the children and handles them most intimately. The other sources of infection, like flies and drinking water, cannot be entirely excluded in the group studied, but it would seem that the evidence is strongest against the mother. Although it is well known that food-handlers are a possible source of histolytica infection, as in the epidemic of amebic dysentery in Chicago reported by Williamson, Kaplan and Geiger (1929), little emphasis has been placed upon this source by most writers, and its importance, especially in the family unit, may have been greatly underestimated.

Inasmuch as this survey was started in the part of Tennessee which was known to have the highest incidence of hookworm, and which appears to have the highest incidence of Ascaris, it may be that the survey has been dealing with the area in which conditions are such as to produce the highest incidence of *E. histolytica* also. If this is so, the present figures are not representative of the incidence for all the rural portions of Tennessee. The present survey is to be continued until all sections of the state are covered, in negro groups as well as whites, so that in the end a true picture of the incidence should be obtained. Ascaris infestation has been shown by Cort, Otto and Spindler (1929) to be closely related to the family unit, and it may be that in rural areas the same may prove to be true of infections with *E. histolytica,* although the factors responsible for dissemination of the two parasites are probably quite different. It is interesting to note that, although the incidence of *E. histolytica* by counties roughly follows that of Ascaris, no correlation between the two parasites has been found in individuals.

Another question which deserves further study is whether *E. histolytica* is always pathogenic, that is, a parasite feeding only on body tissues, or whether it can feed and multiply in the lumen of the intestine as *E. coli* does, without even microscopic damage to the host. If it is always a tissue parasite, then the high incidence in the part of

Tennessee which the survey has studied is worthy of attention as a public health problem.

It is a pleasure to acknowledge the cooperation and assistance rendered in this work by Doctor E. L. Bishop, Tennessee State Commissioner of Health, and members of his staff; by various members of County Health Departments; by Doctor W. W. Cort and Doctor G. F. Otto of the Johns Hopkins Ascariasis Unit; by Doctor A. E. Keller who directed the laboratory; and by the laboratory personnel who made the protozoal examinations and kept the records.

SUMMARY AND CONCLUSIONS

1. A survey now under way in Tennessee has shown an incidence of intestinal protozoa considerably higher than in previous large surveys in this country.

2. The distinctive feature of the survey is that the specimens have come from persons living in their homes, whereas previous surveys (except the recently published one of Faust in Virginia) have dealt with persons in an abnormal environment; hospital, asylum, army camp, etc.

3. This report is based upon the examination of 4987 individuals from 22 counties. Most of the specimens have come from children under 18 years of age, in white rural communities or small towns. Except in the case of special studies only one specimen has been secured from each individual.

4. It has been determined that, with the technique of examination used in this survey, about 50 per cent of the carriers of *E. histolytica* are discoverd by one examination. On this basis of calculation, four counties have shown an incidence of *E. histolytica* between 50 and 56 per cent. The other counties studied have shown a lower incidence, two being between 17 and 20 per cent.

5. The incidence of *E. coli* and *Giardia lamblia* have also been higher than in previous surveys in this country; that of *Endolimax nana* has been lower; that of *Iodamoeba bütschlii* and *Chilomastix mesnili* have been about the same.

6. The calculated incidence of *E. histolytica* in single communities has varied from 92 per cent to 14 per cent.

7. In five families in a backward rural section 86 per cent of the individuals were actually found to be carriers of *E. histolytica,* six examinations being made on most of them. Of the carriers 74 per cent had a history of bloody diarrhea. A low intelligence status and poor personal sanitation characterized these families.

8. Evidence from the histories and stool examinations of these families pointed to the mother as the probable source of spread of *E. histolytica.*

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NEODIPLOSTOMUM GAVIALIS N. SP. FROM THE CROCODILE

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Some 45 specimens of this trematode were obtained in August, 1927, from the duodenum and small intestine of a single crocodile (*Gavialis gangeticus*), about 5 feet long. Afterwards a dozen animals were dissected from time to time, but no other fluke was obtained. Three other species of the parasite are already known from crocodiles but as shown hereafter they differ markedly from this worm. Faust 1927 has described a few Asiatic Holostmos; but he has reported none from this animal. So far as I am aware no trematode of this genus has up to this time been recorded from this country. I am indebted to Mr. S. C. Verma of the Department for valuable help in this study. The type slide is deposited in the Reserve Collection of the Department of Zoology, University of Allahabad.

DESCRIPTION OF THE PARASITE

The worm is 3.5 to 4 mm. in length and 1 to 1.5 mm. in breadth. The measurements represent the mean of five mounted specimens. It has a whitish appearance with two deep brown streaks on the sides, which mark the intestinal ceca. The movements are slow. The body is more or less flattened and divided into two distinct regions, the cephalic and the caudal; the cephalic is 1.8 mm. in length and appears more or less cup-shaped owing to the presence on it of a prominent ventral holdfast organ. The latter lies a little behind the acetabulum, in the posterior third of the anterior body, and bears about 70 papillae on its margin. The oral sucker is small, about 62μ in diameter. The ventral sucker is a little more than twice the size of the oral sucker, being 144μ in diameter. It occupies a position just in front of the hold-fast organ. The caudal region is more or less cylindrical about 1.0 mm. in length, being a little longer than half the length of the cephalic region, from which it is separated by a well marked constriction. Its extremity is truncated and contains the genital atrium. The latter opens to the outside by a fairly wide, terminal opening and two muscular papillae are easily visible within it.

The mouth is terminal and is surrounded by the oral sucker. Directly behind it is the muscular pharynx 54μ in diameter. There is a short esophagus which bifurcates to form the intestinal ceca; these pass on either side of the acetabulum and extend into the caudal region, terminating blindly a little ahead of the posterior end. Their walls are not straight but crenulated.

The testes are large, situated one behind the other about the middle of the caudal region. It is noteworthy that in some specimens a single testis occurs in place of the usual two. The anterior testis (0.369 by 0.288 mm.) is a little smaller than the posterior (0.414 by 0.297 mm.) and somewhat transversely elongated. The anterior vas-efferens arises from the antero-lateral border of the testis and passes as a transverse duct between the ovary and the testis to the opposite side, where it dilates into a seminal reservoir alongside the testis. It then takes a horizontal course running between the two testes, curves backward along the outer margin of the posterior testis, and then bends mediad behind it completing an S-shaped course. Here it is joined by the short vas efferens arising from the second testis and forms a voluminous, slightly convoluted vesicala seminalis. A short duct surrounded by a large number of prostatic cells, arises from it posteriorly and, running through the characteristic bursa, opens in the genital atrium by the side of the muscular papilla. The cirrus is absent.

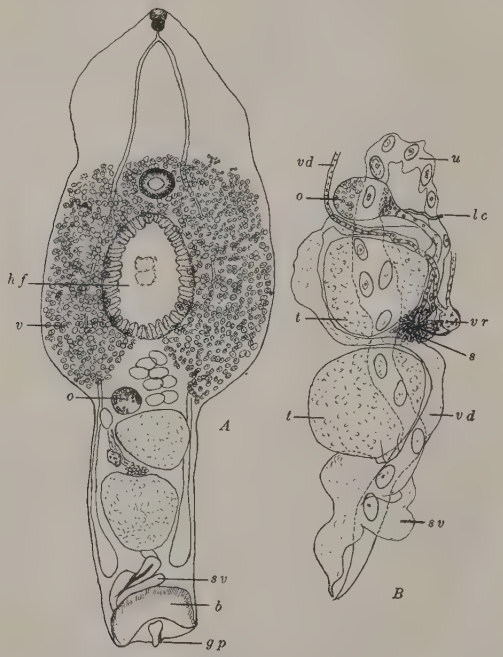
The ovary is dorsal and lies near the commencement of the caudal region of the body. It is oval in shape and measures 0.189 by 0.171 mm. The oviduct arises on one side of it, and is soon joined by the Laurer's canal. It then bends backwards. and receives a short duct from the vitelline reservoir. As it passes through the shell gland which is situated in the space between the two testes, on one side of the median line, it dilates slightly to form the ootype, from which the uterus emerges. The latter at first passes anteriorly and forms a single coil by the side of the ovary, but never enters the holdfast organ. It then turns backwards, and takes a nearly direct course in the middle line to open into the genital atrium. The walls of the uterus are thin, through which the eggs are easily visible. The eggs are few in number and large in size measuring 99 by 77μ. The vitellaria consist of small follicles forming dense clusters. They commence a little in front of the acetabulum and extend over the whole of the posterior cephalic region; but they do not enter the caudal region. A vitelline duct arises on each side and bending backwards enters the caudal region where it meets its fellow of the opposite side to form a short vitelline reservoir lying between the two testes near the median line. A small duct from it goes to the ootype.

There are two principal lateral excretory canals which unite at the posterior end of the body to form a short tubular excretory bladder opening into the genital atrium. The rest of the excretory system is of the usual type.

TAXONOMIC POSITION

The worm is clearly referable to the genus Neodiplostomum Railliet 1919, of the subfamily Polycotylinae, Family Strigeidae (-Holostomidae), La Rue 1926. Apart from the general shape, size and other

characteristics, the new trematode differs markedly from all the ten species, retained in this genus by La Rue, in the disposition of its vitellaria which are confined only to the leaf-like anterior portion of the body. They never extend into the caudal region as they do in the other known forms. In possessing a holdfast organ with a margin produced into prominent papillae it resembles *Diplostomum spathulaeforme* Brandes, *Diplostomum siamense* Poirier, *Diplostomum longum* Brandes and *Diplostomum spathula* Brandes, and differs from other



Text Figure. A. *Neodiplostomum gavialis* n. sp. Ventral view × 60. B. Semi-diagrammatic representation of the genitalia. *b,* bursa; *gp,* genital pore; *hf,* hold fast organ; *lc,* Laurer's canal; *o,* ovary; *s,* shell gland; *sv,* seminal vesicle; *t,* testis; *u,* uterus; *v,* vitellaria; *vd,* vas deferens; *vr,* vitelline reservoir.

species. The number of papillae in my species is about 70 while in *D. siamense* it is 40, and in *D. longum* 20; but in *D. spathula* and *D. spathulaeformae* the number is much greater. The relative sizes of the cephalic and the caudal regions afford another important character for further distinguishing the new species from the allied forms. In *D. longum* the hind body is twice as big as the fore body; in *D. siamense* and *D. spathula* the two regions are equal to one another;

in *D. spathulaeforme* and the species under consideration the fore body is larger than the hind body but the former is a much smaller form, and differs in characteristics already pointed above. The third species from the crocodile, *D. bifurcatum,* is also distinguishable by its much larger size, by the absence of papillae on the holdfast organ, and by its peculiar caudal portion which is larger than the cephalic and divided into two lobes.

Thus *Neodiplostomum gavialis* n. sp. stands clearly apart from the other known species of the genus in several very important features.

DIAGNOSTIC CHARACTERS

Neodiplostomum: Length 3.5 to 4 mm., breadth 1 to 1.5 mm. Cephalic region lamellar, less than twice as long as caudal. Oral sucker terminal; pharynx almost as large as oral sucker; esophagus short. Ventral sucker more than twice as big as oral sucker, in front of middle of cephalic region. Holdfast organ prominent, behind middle of fore body and about one-third its length; margin with 70 papillae. Ovary small, rounded, at junction of cephalic and caudal regions. Testes large, behind ovary; vesicula seminalis prominent, behind testes. Prostate present. Vitellarian follicles small, densely packed, from in front of ventral sucker to end of fore body. Uterus simple, never reaching holdfast organ, with few large eggs 99 by 77μ. Bursa with two papillae. Genital opening terminal.

Host: *Gavialis gangeticus.*

Location: Duodenum and small intestine.

Locality: Allahabad, India.

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SOCIETY PROCEEDINGS

HELMINTHOLOGICAL SOCIETY OF WASHINGTON

One Hundred Twenty-First to One Hundred Twenty-Sixth Meetings

The one hundred twenty-first meeting was held May 18, 1929.

Dr. N. A. Cobb presented notes on the effects of blasting and electric currents on soil nemas and on the excretory function of the skin, with a demonstration of cups made from nickel wire cloth for use in studying soil nemas. (To be published elsewhere.)

Mr. W. E. Dove presented a note on creeping eruption in man. (To be published elsewhere.)

Miss Myrna Jones presented the following: 1. A new tapeworm from the guinea fowl, with cysticercoids in a ground beetle.—Numerous tapeworms collected from the viscera of two guinea fowl (*Numida meleagris*) obtained from the market in Washington, D. C., were found to belong to the genus Raillietina, subgenus Paroniella. They are in many respects similar to *R.* (*P.*) *numida* Fuhrmann, 1912, but appear to differ in such fashion as to be considered a new species under the following name:

Specific description: Raillietina (Paroniella) magninumida: Length 100 to 150 mm., maximum width, 1.3 mm., scolex 160 to 210μ in diameter; suckers 50 to 65μ in diameter, and armed with numerous rows, at least 10, of small hooks. The rostellum is armed with approximately 150 to 160 hooks, 8 to 10μ long, arranged in a double crown. The cirrus pouch is very large, 280 to 350μ long, and the cirrus is heavily armed, the larger hooks being 22 to 24μ long and the smaller 18 to 20μ long. The testes vary in number from 13 to 18, the usual number being 15. The female genital complex is similar to that of *R.* (*P.*) *numida* except in the matter of size. This material differs from *R.* (*P.*) *numida,* which has been reported only from Africa, in being larger in all respects except for the size of the hooks of the rostellum and suckers. The hooks of the cirrus are markedly larger. The number of testes reported for *R.* (*P.*) *numida* is 7 to 10, while that of the species in question in no case had as few as 10. Should intermediate stages be found, the description of the earlier species might be changed to include such variation, but for the time being it seems wiser to consider the new material as a distinct species.

Gravid segments of this material were fed to the ground beetle *Selenophorus pedicularius* and after three weeks cysticercoids were found with scolices morphologically similar to those of the original material. No infestation resulted from feeding the cysts to chickens or quail, so that complete evidence for the life cycle is lacking, though perhaps failure to produce infestations in unusual hosts should not be regarded as highly significant.

2. The ground beetle *Selenophorus pedicularius,* which has been previously reported as carrying immature cysticercoids of *Raillietina* (*Skrjabinia*) *cesticillus,* has been found to serve as an intermediate host for that species; similarly has the dung beetle *Aphodius granarius,* previously known as a host for another poultry tapeworm, *Hymenolepis carioca.* These results are based on experimental feedings of the cestode material to beetles, cystercercoids having been found in the beetles after approximately three weeks, and tapeworms having been recovered from chicks that were given the infested beetles. Control birds remained free from the cestode in all cases.

Cysticercoids of *R.* (*S.*) *cesticillus* have also been found in the ground beetles *Selenophorus ovalis* and *Triplectrus rusticus,* but the final work of infecting chickens with the material has not been completed. The two species of cestodes

mentioned are the ones most commonly found in routine examinations here, and it is interesting to note these indications of an extensive list of intermediate hosts. Several cysticercoids, probably of *Choanotaenia infundibulum,* were found in a ground beetle, *Cratacanthus dubius,* collected from a chicken yard near Crete, Nebraska, during the summer of 1928. In general structure, and particularly as to the hooks of the rostellum, the cysts correspond closely to the description by Guberlet of the cysticercoid of *C. infundibulum* found in the house fly. Only negative results were obtained, however, from feeding this material to a chicken, so that evidence that this beetle actually serves as an intermediate host is not complete. It is known that chickens in this yard have been infested with *C. infundibulum,* and it is hoped that more material may be obtained.

3. The following are new host records for bird cestodes, and are also reported from this country for the first time, so far as can be determined: *Cladotaenia globifera* in *Circus hudsonius,* a marsh hawk brought in to the laboratory from near Washington, D. C., and in *Asio flammeus,* a shorteared owl collected by E. E. Wehr from near Miles City, Montana. *Hymenolepis (Echinocotyle) rosseteri* was collected by Eloise B. Cram from a shoveler duck, *Spatula clypeata,* sent in from New Mexico. In addition, the fowl cestode *Hymenolepis cantaniana* is reported from the quail, *Colinus virginianus,* collected by the Biological Survey in Maryland.

Dr. L. A. Spindler presented a note on the relation of moisture to the distribution of human Trichuris and Ascaris.—In connection with studies on the epidemiology of ascariasis in southwest Virginia during the summer of 1928, it was found that the Trichuris incidence was much lower than that of Ascaris except in a few areas where the ground was damp due to the presence of heavy shade. A survey of the literature revealed a similar situation in other parts of the world, the Trichuris incidence being less than that of Ascaris in regions of light rainfall and equal to or higher than Ascaris in regions of heavy rainfall.

The results of laboratory experiments indicated that Trichuris eggs are less resistant to desiccation than those of Ascaris, perhaps accounting in part for this peculiarity of distribution.

Dr. G. Steiner presented notes on 1: The occurrence of *Tylenchus similis* on new host plants, and 2: The effects of *Tylenchus dipsaci* infestation of oats.

Dr. E. W. Price presented the following: *Wellcomia compar* (Leidy) the correct name for *Oxyuris compar* Leidy, 1856.—In 1856 Leidy described an oxyurid from the small intestine of a cat for which he proposed the name *Oxyuris compar.* Hall (1922) placed this species as a synonym of *Oxyuris ambigua* (= *Passalurus ambiguus*), his conclusion being based on the description of the worm as given by Leidy to the effect that the tail was spirally contorted, a condition which might possibly have been confused with the annulate condition of the tail of *O. ambigua,* and also upon the fact that the latter species is a more or less common parasite of rabbits and one which might have been taken into the digestive tract of the cat by ingestion of viscera containing the parasite. This hypothesis is supported by the fact that oxyurids are not known to occur in cats. Recently the writer located a few specimens of females of *O. compar* in the Helminthological Collection of the United States National Museum, this material being a part of the original Leidy collection. Examination of these specimens show that they belong to the genus Wellcomia Sambon, 1907. This species appears to be very closely related to *W. mitchelli* Sambon, 1907, from the Cape jumping hare. The normal host for *W. compar* remains a mystery, but it is probably some wild mammal, perhaps a rodent of some sort.

The one hundred twenty second meeting was held September 19, 1929.

Mr. B. G. Chitwood presented a note on the excretory system of an oxyurid closely related to *Thelastoma leukarti* (Hammerschmidt, 1838). (To be published elsewhere.)

Dr. N. A. Cobb presented the following note:

The Demanian vessels in female nemas of the genus Oncholaimus.—Continuing the observations of de Man, 1884, and zur Strassen, 1896, observations have been

made on *Oncholaimus fuscus* Bastian, *O. pristiurus* zur Strassen and half a dozen other marine Oncholaims with particular reference to the system of tubular organs discovered by de Man. Building on the foundation laid by these eminent observers, it has been possible to define the Demanian System and assign it a function. The following definition and table of homologous terms, together with the accompanying text appreciably advance the knowledge of this remarkable system of organs.

Demanian Vessels are a complicated double system of efferent tubes in adult female nemas (Oncholaims) connecting, (1) with the middle or posterior part of the intestine, and (2) with the uterus (or uteri); these two efferents are confluent at a special glandular "gateway," the uvette, and then empty backward and outward, through one or two ducts having more or less moniliform affluent glands. The ducts lead to pores in the body wall, usually laterad, one or more on each side, near the base of the tail. The demanian vessels elaborate a copious, elastic, sticky, non-watersoluble secretion presumably utilized to protect and preserve the batches of eggs after deposition and during segmentation.

Equivalent Terms of Various Authors

De Man	*Zur Strassen*	*Present*
Hauptrohr	Stammrohr	Enteric Efferent
Verbindungsröhr Zwischen Warze und Uterus	No mention	Uterine Efferent
Warze	Rosette	Uvette
Rothbraunnen drüsen	Endschlauch des Rohrorgans	Moniliform Glands
Blindes Vorderende	Mundungsorgan	Selective Demanian intake (enteric)
Ausführungsgang in den Uterus	Blindgeschlossener Seck	Demanian Intake (uterine)

The demanian organs seem to prevail in mud-inhabiting, and sand-inhabiting Oncholaimus, and to be absent or less prevalent in the Oncholaims living in more thoroughly oxygenated water, e. g., on the surface of eelgrass, and among algae.

It is interesting that the females of nearly all Oncholaims thus far shown to possess demanian vessels are more or less subject to uritis,—disease of the caudal extremity. While these diseases are of considerable variety, a number of them have the point in common that they attack particularly the posterior portion of the female. In one case from a given locality, not a single healthy female could be found. The disease consists in an outbreak of pustules on the tail and posterior part of the nema, but particularly on the tail, which finally results in a necrosis that, proceeding cephalad, may destroy a very considerable portion of the nema, even as far forward as the vulva.

An interesting morphological problem is thus disclosed. One finds Oncholaims, especially females, with highly peculiar caudal extremities,—sometimes without spinneret or anus, sometimes with these organs present but in an abortive or peculiar form. Examination of a number of Oncholaims of this character convinces me that often these abnormalities are due to a specific disease.

Oncholaims inhabiting mud and sand are especially subject to disease. Among these diseases there are one or more characterized by attacking the tail end of the nema only—uritis—and reducing it to a necrotic condition. The disease is sometimes combated by the nemic organization, so that the posterior end of the nema heals over, and in some cases it seems as if a new anus is formed, and possibly even a new spinneret. Just how this occurs is not yet clear, but I have seen both

deformed anal openings and deformed spinnerets of female Oncholaims that gave evidence of having been imperfectly regenerated after some accident, or what is far more likely, after uritis and attack of one of the diseases just mentioned.

Among Oncholaimus thus attacked, allusion is made to the following cases: (1) A "Tailless" form from Woods Hole, Massachusetts, U. S. A. This is undoubtedly a case of healing over. (2) I have seen numerous specimens of a new and undescribed genus of Oncholaims with the tail end in a necrotic condition;—sometimes nearly half the nema consumed by the disease. I have never seen any regeneration in this species. (3) I have seen a specimen of *Oncholaimus pristiurus* or what was believed to be such (it was found in a collection in which *pristiurus* was numerous, while no other Oncholaim was found in the collection), the posterior extremity of which was healed over in front of the anus, leaving no anal opening and no trace of a spinneret. (4) I have quite frequently seen specimens of *Oncholaimus fuscus* presenting the condition described. In one or two cases, especially in specimens sent me by Miss E. Horsman, University College of Wales, I reported that if the organization normally was that presented in the specimens sent, they would represent a new species, but that I could not be sure they were not specimens which in some way had been mutilated. At that time, I knew very little about diseases affecting Oncholaims. I am now satisfied that these Welsh specimens were specimens that had become diseased at the tail end, and had afterward "recovered." (5) I have seen a slender new Oncholaim at Woods Hole diseased at the posterior end, although I do not recall any cases of healing over. (6) My files contain a description of another, presumably new Oncholaimus from Florida, which probably also is an example of healing over. In this case there was formed an imperfect spinneret and anal opening. I feel certain I have not mentioned all the cases of this character I have observed.

Dr. G. Steiner exhibited a piece of pine wood from a pole in the yard of the Texas Creosoting Company. The wood showed dark streaks between the parts with the bluish stain. These streaks are inhabited by numerous specimens of an Aphelenchus species. (To be published elsewhere.)

Dr. Steiner also reported the finding of a new nemic parasite of Japanese beetle larvae. The specimens were from Dr. Glaser of the Rockefeller Institute for Medical Research, Princeton, N. J., and sent in for identification. They belong to the Oxyuridae and apparently to a new genus and a new species, most closely related to the genera Aplectana and Steinernema. A detailed account will be given elsewhere.

Dr. E. W. Price presented the following notes:

1. Some malformations in trematodes.—During the past year two cases of monorchism were encountered in trematodes, one in *Phyllodistomum fausti* Pearse and the other in *Echinostoma* (?) *revolutum*. In both instances the missing testis was represented by a small mass having no resemblance to testicular tissue. Monorchism appears to be one of the most frequently encountered malformations in trematodes, but such cases are by no means common. A third case of malformation which appears to be unique was observed in a mature specimen of *Fasciola hepatica*. This specimen showed two apparently normal, functional ovaries. The specimen appeared normal in all other respects.

2. The occurrence of *Trichostrongylus rugatus* Monnig in a mountain sheep.—A heavy infestation with *Trichostrongylus rugatus* was recently encountered in a young mountain sheep, *Ovis canadensis*, which died in the National Zoological Park. This species was described by Monnig in 1925 from South African sheep but up to the present it has not been reported in this country.

3. Thaumatocotyle Odhner, 1910, a preoccupied name.—The new name Braunotrema is proposed to replace Thaumatocotyle Odhner, 1910 (Trematode) not Thaumatocotyle Scott, 1904 (Trematode). The type species is therefore *Braunotrema pulvinata* (Braun, 1899).

4. Plagitura Holl, 1928, a synonym of Manodistomum Stafford, 1905.—In comparing the type specimens and description of *Plagitura salamandra* Holl, 1928,

with the description of *Manodistomum occultum* Stafford, 1905, the writer is convinced that the two species are identical in spite of the fact that Stafford's description is based upon immature material and, therefore, incomplete in some respects. The position of the testes with respect to the termination of the intestinal ceca, together with the fact that the specimens were obtained from the same host, make it unlikely that the two forms are distinct species.

The one hundred twenty third meeting was held October 19, 1929. The following officers were elected for the ensuing year:

President, Dr. Paul Bartsch.

Recording Secretary, Mr. J. R. Christie.

Corresponding Secretary, Miss Myrna F. Jones.

Dr. G. Cassidy reported as follows: In July, 1929, at Malang, Java, a brief survey was made of nematode infested material obtained from neighboring coffee plantations. A wide range of genera was found including two species of Tylenchus,—namely *Tylenchus coffeae* and *Tylenchus acutocaudatus,* originally described by Zimmerman, 1898. Microscopic examination shows that *Tylenchus acutocaudatus* is synonymous with *Tylenchus similis* described by Cobb in 1892. En route to Java various sugar growing countries were visited and cane roots and soils were examined for the presence of nematodes. *Tylenchus similis* was found in cane roots in the island of Negros (Philippine Is.), in Java, and in South India. In Formosa cane and soils were examined after a drought of five months and *Tylenchus similis* could not be demonstrated at that time. Dr. Miyake, plant pathologist, Taihoku, reports, however, that *Tylenchus similis* is found abundantly in cane during the wet season. The following is a list of known hosts of *Tylenchus similis:* banana, sugar cane, nut grass, pigeon pea, coffee, canna.

Dr. Ena A. Allen presented the following notes: Recently, on a quail farm near Richmond, Virginia, there has been a severe epizootic among quail which have been artificially raised. Since they were dying in great numbers, sometimes over one hundred in a day, some of them were sent to the Bureau of Animal Industry for diagnosis. Of those a few died on the way. Autopsies were made of them and of some of the sickest birds which we killed. In every quail examined the ceca were very heavily infected with Trichomonas. These resemble *T. gallinarium* of the chicken; the species, however, has not yet been determined. Perhaps the invasion of this Trichomonas into the cecal walls made sufficient lesions for the bacteria to enter the blood stream. Dr. Shook of the Pathological Division has made quite a number of cultures from the heart blood, spleen, and ceca of birds just killed. In each case he found *Bacillus coli* in the heart blood. Fresh smears from the heart revealed a very few red blood corpuscles, all of which were degenerate, and many bacteria. This Trichomonas was successfully transmitted to chickens and the same conditions were found in them as in the quail. These findings suggest that this Trichomonas in sufficient numbers is pathogenic for quail and chickens.

Dr. G. Dikmans presented the following: 1. *Ostertagia lyrata* first reported by Sjoberg from cattle in Europe in 1926, was found in cattle belonging to the Iberia Livestock Experiment Farm, Jeanerette, La., in the same year. It was identified by Dr. E. W. Price of the Zoological Division, Bureau of Animal Industry, in 1927 from specimens sent from Louisiana. 2. Two specimens of *Fasciola hepatica* were found in the bile ducts of a rabbit at the Zoological laboratory at Jeanerette, La. This fluke, a common parasite of cattle in the coastal plains region of Louisiana, has been reported as a parasite of rabbits and hares in Europe but as far as the writer is aware, this is the first report of its occurrence in these animals in the United States.

Mr. Joseph E. Alicata presented the following note: Up to the present time the occurrence of *Globidium* in the abomasum of American sheep has not been reported. The late Dr. B. H. Ransom observed these parasites in American sheep several years ago and communicated his finding orally to Dr. Maurice C. Hall. Unfortunately he failed to publish this record. On July 26, 1929, 7 out of 78

sheep which originated in Purdue University, Purdue, Indiana, and were slaughtered at the U. S. Experiment Station at Beltsville, Maryland, showed the presence of these parasites. They appear as small whitish nodules located in the mucous lining of the abomasum, each nodule measuring about 1 mm. in diameter and being raised above the surface of the mucosa to a height of about 0.5 mm. The cyst which is inclosed in the nodule is more or less elliptical in shape and from 450 to 700μ in length and 325 to 465μ in width. The contents of each cyst are made up of a mass of crescent-shaped sporozoites, 5.5 to 7.5μ long and 1.5 to 2μ in maximum width. When stained with iron-haemotoxylin, the nucleus becomes readily discernible. It is elongated and measures about 2μ in length and about 1μ in width. Similar cysts were observed in sheep slaughtered at Beltsville on September 11, 1929, 11 out of 101 sheep killed, being infested. On October 10, 1929, 6 out of 72 sheep killed were also infested with these protozoa. The origin of these sheep in the first case was West Virginia, and in the second case they came from Idaho.

Dr. C. W. Stiles and Mrs. C. E. Baker reported that among the numerous questions on nomenclature recently sent to the Hygienic Laboratory for expression of opinion was one on nematodes submitted by Dr. E. B. Cram of the Society. This involved the generic names Tetrameres, Tropisurus and Tropidurus in regard to which some confusion exists at present in Helminthological literature. A study of the original documents demonstrates that Wiegmann, 1835, and Agassiz, 1846, were entirely in the right in emending Tropisurus Diesing, 1835, to Tropidurus. The name Tropidurus is, however, preoccupied by Tropidurus Neuw., 1824, reptile. Accordingly, it is a dead homonym. Had Tropisurus been proposed as an arbitrary combination of letters it could stand in spite of Tropidurus, 1824, but it was proposed with definite citation of its derivation and, therefore, comes under Article 19 of the International Rules. *Tetrameres* Creplin, 1846, appears to be the oldest available name for the nematode in question. It is not clear why Baylis & Daubney, 1926, revert to *Tropisurus*.

Mr. B. G. Chitwood presented the following description of a new nema parasitic in the intestine of Fontaria. Two specimens were found in the intestine of a milliped, identified by Dr. Kock, Bureau of Plant Industry, as *Fontaria coriacea*, which was collected in a wood not far from the chain bridge leading to Washington. They are members of the family Oxyuridae. In the literature available to me I was unable to find any genus in which it would fit. Therefore, I am making of it a new genus.

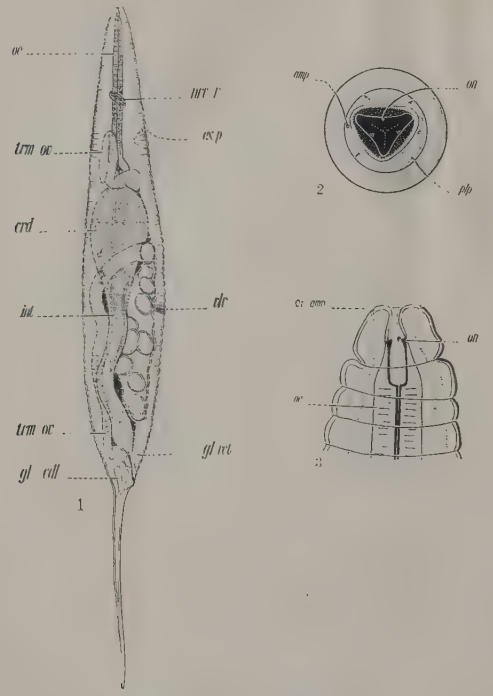
Fontonema n. g. The genus may be characterized as having three lips and three intermediate thickenings of the cuticula of the pharynx wall, forming small teeth. The first striation is deep, simulating a head. Esophagus, typical oxyurid; two ovaries, each being reflexed at its opening into the two-horned uterus so that the ovary which originates from the anterior horn of the uterus lies outstretched in the posterior portion of the body while the ovary which had its origin in the posterior horn of the uterus lies outstretched in the anterior part of the body, and is reflexed at its blind end.

Fontonema brachygaster n. sp. Length 0.62 —0.84 mm.

3.	11.	25.5	45.	67.	0.62 —0.84 mm.
4.	5.6	8.7	13.6	5.5	

Measurements taken on two females, both adult, fixed in hot alcohol and mounted in glycerine jelly. All measurements were taken under some pressure. No males were found. The three lips are equal. The dorsal lip has two papillae near its outer margin; each of the subventral lips have a lateral organ or amphid and a subventral papilla. An amphidial pouch is obvious in side view but terminals were not visible. The lips are not separated by deep indentations. The pharynx extends to the middle of the second segment. Onchia are three, equal, small thickenings of the cuticle best seen in side view as they are somewhat covered in face view. The nerve ring is located slightly anterior to the middle of the esophagus (10-12% of the body length from the anterior end). The excretory pore

is quite distinct, located at a latitude of about twenty. No longitudinal ducts were seen but a reservoir seemed to be present. The cuticular striations are very coarse, but they disappear at a point even with the anus. There are 70-75 such annulations in the length of the body. The esophagus and tail make up quite a large proportion of this nema, the former 20, the latter 30 per cent. The anterior ovary extends past the cardiac esophageal bulb and is reflexed at a point about even with the excretory pore. Definite anal glands were seen as were also caudal glands. The tail is long, thin and tapering.



Text figure. *Fontonema brachygaster.* 1. Adult female, × 145; 2. Head in end view, × 325; 3. Head in side view, × 325.

Miss Myrna F. Jones presented the following notes: 1. Gravid material of *Davainea proglottina* was fed to the common pond snail, *Physa heterostropha.* After 18 days one of the snails was found dead with at least 25 apparently mature cysticercoids of *D. proglottina* in its tissue. The material, though not active, was fed to a chick, with negative results upon post-mortem examination; however, Physa might be considered, with this incomplete evidence, as a probable intermediate host, but an unusual one.

2. Based upon experimental feedings, the ground beetle (*Cratacanthus dubius*) was found to serve as an intermediate host for the cestode, *Raillietina* (*Skrjabinia*) *cesticillus.* A chick given this beetle, containing cysts, and examined after 5 days

was found to harbor several young specimens of *R.* (*S.*) *cesticillus*. Control chicks were negative for all cestodes.

3. Numerous cysticercoids, typically hymenolepid and with unarmed scoleces, were found as natural infestations in two specimens of the dung beetle, *Onthophagus hecate*, collected from the poultry yards at the U. S. Experiment Station at Beltsville, Maryland. Two chicks were fed this material and upon post-mortem examination each chick contained specimens of *Hymenolepis carioca*. One examined after 25 days harbored mature tapeworms, and in the other, examined after 11 days, the specimens had no gravid segments, but were about 40 mm. in length. The seven control chicks of this lot were negative of all helminths. In the collection at this laboratory there is a record of hymenolepid cysts from *Onthophagus hecate* collected by Dr. Ransom at Bloomington, Illinois, in 1920. These had been labeled as *H. diminuta* (?). Since there is no record of experimental feedings, and comparison shows that this material is very similar to that recently collected from the same host, it seems probable that this material is also *H. carioca*. I am indebted to Dr. Marshall of the U. S. Museum for identification of the snail, and to Dr. Chapin and Dr. Buchanan of the Bureau of Entomology for identification of the beetles.

The one hundred and twenty-fourth meeting was held Nov. 16, 1929, at the School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.

Dr. C. Davis Sherborn was elected an honorary member.

Dr. Justin Andrews presented a note on the use of Zebe-a-pique as a malariacide in Grenada, B. W. I. (Details will appear in the American Journal of Hygiene.)

Dr. G. Steiner discussed the nemic fauna of slime flux of the Carolina poplar. (Details to appear elsewhere.)

Dr. J. H. Sandground summarized as follows the helminthological findings of the recent Yucatan Medical expedition of the Department of Tropical Medicine, Harvard Medical School, in conjunction with the Carnegie Institution. Investigations were carried out at Merida, at the Carnegie Archaeological Station at Chichen Itza and other rural centers.

The country is of lime-stone formation, nearly level in topography and the rainfall is seasonal and sparse. Except in the interior there are no rivers, lakes or marshes, hence aquatic animal life, including molluscs, is poor and trematode infections are rarely encountered. No evidence was obtained to confirm the reports of endemic human paragonimiasis and schistosomiasis that have been made. Ascariasis is the commonest human helminth infection, up to 100% in some rural communities. Trichuris is also very common. Both the incidence and intensities of hookworm infestations are low. The only hookworm found was *Necator americanus*. Enterobius infestation was exposed very frequently by treatment with anthelminthics. *Taenia solium* infection is abundant and cases of *Hymenolepis nana* and *H. diminuta* were also found.

Cattle, pigs and dogs were among the domestic animals examined. In cattle the only strongyle found belonged to a new genus and species. Specimens were demonstrated to members of the society. Cysticercosis in cattle is very rare. On the other hand, *Cysticercus cellulosae* is common in the pig. A very heavy natural infestation with *C. cellulosae*, apparently the first to be reported from the Americas, was also found in the dog. The commonest parasite of the dog is *Spirocerca sanguinolenta*. Descriptions of several interesting new parasitic nematodes from a bat, buzzard and iguana will soon appear.

Mr. B. G. Chitwood summarized the results of his study of the esophagus of the Trichinelloidea as follows: An examination of toto mounts of adult *Trichinella spiralis* and of transverse sections shows that the esophagus is not fundamentally different from that found in other nematodes. The anterior portion near the head is quite small but shows radiating fibers. The nerve ring, showing posterior ganglion cells, surrounds this portion. Posterior to nerve ring the esophagus forms a pseudo-bulb without valves. Immediately anterior to the cell body it narrows abruptly and bends ventrally and to one side, extending posteriad

to the intestine. It may cross the cell body or bend around it; in any event it does not hold a constant position, but may be seen ventrally, sub-ventrally, dorsally or sub-dorsally. It generally enters the intestine from a dorsal position, bending ventrally in a short curve. The esophagus lies in a groove or gutter in the cell body, but is rarely imbedded to a depth of more than half its diameter except at its posterior end where it bends ventrally to join the intestine. Here, for a very short distance it is completely surrounded by the cell body. Both the esophagus and cell body are each inclosed in a membrane, and in no case does the esophagus lie next to the cell body without intervening membrane. The lumen is triradiate throughout. Radiating muscles probably exist in all parts of the esophagus, varying from mere suggestions in the small tube opposite the cell body to distinct muscles in the pseudo-bulb. Large, distinct nuclei may be seen in the bulb while in the esophagus parallel to the cell body minute bodies are present which probably are also nuclei. The cell body appears to be an enormous outgrowth from the intestine which nearly fills the anterior portion of the body and may be likened to a cecum without a lumen.

In the case of *Trichuris ovis* and *Trichuris vulpis* the portion of the esophagus anterior to the cell body closely resembles that of *Trichinella spiralis*. On reaching the cell body the esophagus turns abruptly to one side and is from this point posteriad, imbedded in the cell body. It varies somewhat in size and in some places the muscle layer is but weakly developed, allowing the lumen to open and appear circular or take on other odd shapes when seen in cross section, but it is fundamentally triradiate throughout, for one can distinguish joints in the cuticular wall.

Near the posterior end of the cell body, two other unicellular bodies appear, and in this region the esophagus comes out of the cell body to some extent where it enlarges, possesses a flat lumen, and forms an intestinal valve similar to that of *Trichinella spiralis*, a peculiar half-moon shaped affair around which the anterior part of the intestine is seen. The cell-body projects 12-25μ posterior to this valve and little evidence was obtained in this case to indicate whether it was of esophageal or intestinal origin. The esophagus of *Trichosomoides crassicauda* is intermediate between the type found in Trichuris and that of Trichinella but more closely resembles the latter.

Conclusions.—The esophagus of Trichinella, *Trichosomoides* and Trichuris does not differ fundamentally from the typical nematode esophagus. Each possesses a lumen fundamentally triradiate throughout, surrounded by a muscle layer and enclosed in a membrane. The esophagus may be pressed against the cell body and slightly embedded therein (Trichinella) or completely embedded (Trichuris), but always separated from the protoplasm of the cell body by a membrane. The peculiar structure variously called the Zellenkörper, Zellenschlauch or cell body, is probably not an esophageal structure, but of intestinal origin. The present conception of the esophagus of the Trichinelloidea which has permeated the literature and textbooks, and upon which this taxonomic group is primarily based; namely, that it is a row of cells pierced by a narrow, possibly circular tube with a cuticularized wall, is erroneous. Further details will be published elsewhere.

Dr. N. A. Cobb gave further notes on the Demanian system of vessels in female nemas of the genus Oncholaimus. He also discussed mermithid and other nemic parasites of ants.

Mr. J. E. Stumberg presented a note on the antigenic properties of infective larvae and adults of the dog hookworm, *Ancylostoma caninum*. (Details will be published elsewhere.)

Mr. Eugene Schumaker discussed experimental infection of the rat with the *Balantidium* from the domestic pig. (Details will appear elsewhere.)

Dr. F. M. Root reported on recent studies of the mosquito fauna of the island of Grenada, B. W. I.

The one hundred and twenty-fifth meeting was held December 21, 1929.

The following note by E. M. Nighbert of the U. S. Bureau of Animal Industry field station at Moultrie, Ga., was read: On July 19, 1929, fresh samples of urine from eight sows were collected and examined for the presence of kidney worm ova. The specimens of urine from three of the animals were negative; three were positive, showing evidence of kidney worm infection in the form of eggs present. In one of the positive samples of urine, in addition to the casts and flocculent material present, one almost complete oviduct filled with eggs was observed. This finding is further evidence that the kidney worm, as well as its eggs, can and does leave its natural location in the kidneys or kidney fat of the host animal, and passes out through the urinary tract. A previous report of a similar observation was made by Nighbert in the Journal of the American Veterinary Medical Association (73 (7): 874-875).

Dr. M. C. Hall presented a short note entitled: Are the metastrongyles strongyles? On the basis of musculature, lip structures, larval structures, and habits, the metastrongyles are regarded as probably spirurid in their relationships rather than strongyle, the atypical metastrongyle bursa being regarded as misleading.

Dr. N. A. Cobb presented two notes: (1) Nemas associated with ants. (2) Chromatropism in nemas.

Miss Mary S. Skinker reported on a new species of nematode of the genus Cystidicola from fishes. Numerous specimens of nematodes from the swim bladder of *Leucichthys artedi*, and a few from the intestine of *L. hoyi*, and also some from the swim bladder of *L. reighardi* were sent by Mr. Andrew L. Pritchard of the Pacific Biological Station, Nanaimo, British Columbia, to the Bureau of Animal Industry for identification. They were collected in the vicinity of the Bay of Quinte, an inlet of the northern shore of Lake Ontario. They belong to the genus Cystidicola and are provisionally considered a new species for which the name *Cystidicola canadensis* is proposed. The characters which separate this form from other species in the genus are the size of the specimens and the presence of eight small teeth in addition to the two large teeth found in *C. farionis*. The mature females measure 30 to 51 mm. in length, the males 25 to 40 mm. The preanal double papillae situated within the alae vary in number, usually approximating six or seven pairs. Five pairs of postanal papillae are present, and the posterior pair of these is double. The spicules are dissimilar, the long one 840 to 870μ in length, the short one 210 to 230μ. A detailed description of this form is to be published elsewhere.

The following notes by E. E. Wehr were read:

(1) The occurrence of *Ornithostrongylus quadriradiatus* in the mourning dove.—At the suggestion of Dr. M. C. Hall I examined a number of mourning doves in the vicinity of Fort Keogh, Miles City, Montana, with the idea that a small nematode which is known to be pathogenic to the pigeon in the United States (Washington, D. C.) and Australia might occur in these birds. After sixteen birds had been autopsied and the intestinal contents carefully washed and screened two males and five females were recovered, all of which were blood-red in color. A microscopic examination of these nematodes was made and they were determined as the species which was found by Stevenson (1904) and later by Cram to be present in pigeons in Washington, D. C., namely *Ornithostrongylus quadriradiatus* (Stevenson, 1904) Travassos, 1914. On account of their small size, these nematodes are quite easily overlooked unless care is exercised in looking over the contents of the intestinal tract.

(2) *Fimbriaria fasciolaris* as a parasite of the mallard in the United States.—This small and interesting cestode was collected in small numbers from the intestines of mallard ducks killed on Lake Garberson, a small artificial lake on the United States Range Livestock Experiment Station, Miles City, Montana. There appears to be no record of this tapeworm occurring as a parasite of the mallard duck in the United States, although it has been reported by several writers from various other birds belonging to the order Anseres.

(3) The genus Tatria recently found in the United States.—During the month of May two eared grebes (*Colymbus nigricollis californicus*) were killed on Lake Garberson, Miles City, Montana. The intestines of these birds contained hundreds of small cestodes belonging to the genus Tatria, the species of which has not yet been determined. The proglottids, which are few in number, carry lateral digitate processes. So far as the writer is able to ascertain, this genus of cestodes has not been reported from this country. It has been recorded as occurring in the horned grebe in Europe.

(4) A nematode pathogenic to the sage grouse.—Underneath the tunic lining of the gizzard of a bird killed October 18 on the United States Range Livestock Experiment Station, Miles City, Montana, there were found two nematodes, a male and a female, rather large in size and red in color. They were determined as belonging to the genus Habronema, but the species is still in doubt. On October 26, two more birds were killed at a point 18 miles from the station on Moon Creek. There was discovered, on separating the horny lining of the gizzard from the muscular coats of one of them, in the region of the union of the proventriculus and the ventriculus, a large number (108) of blood-red nematodes. This heavy infestation had induced a necrotic condition which extended over an area including the anterior one-fourth of the ventriculus, part of the proventriculus and the constriction between these two organs. Large numbers of the worms were found between the horny lining and the muscular coat of the gizzard, a few were found on the inside of the horny membrane, and many were sewed back and forth through the dead tissue.

Mr. B. G. Chitwood discussed the histology of the excretory system in *Macracis monhystera*. (Details to appear elsewhere.)

Dr. Benjamin Schwartz presented the following note by himself and Mr. J. E. Alicata on the occurrence of encysted nematode larvae in the liver and lungs of swine. Several livers and lungs of hogs, forwarded to the Bureau of Animal Industry from Madison, Wisconsin, for diagnosis of lesions, showed the presence of small pearly cysts beneath the pleural covering of the lungs and under the capsule of the liver. The cysts are more or less transparent, globular in shape, and from 1 to 2 mm. in diameter. Nearly every cyst from the lung which was examined contained a partially calcified nematode larva; those from the liver which were examined did not contain larvae. One larva was found to be 1.7 mm. long and 62μ in maximum width; the tail measured 92μ. The anterior end of the larva is bluntly rounded and shows 3 lips. The details of the internal morphology could not be made out. The tail has a dorsal direction, tapers gradually, its tip being bluntly rounded. So far as concerns size, shape and external morphology, these larvae correspond to the stage of *Ascaris lumbricoides* which has been described from the lungs. The liver shows considerable mottling due to an increase in interlobular connective tissue, these lesions being very pronounced in the vicinity of the cysts. The pathological picture of the liver associated with these parasites shows a diffuse, superficial fibrosis, whereas that produced by Stephanurus shows large areas of fibrosis extending deeply into the liver tissue.

The one hundred and twenty-sixth meeting was held January 18, 1930.

The Society listened to an interesting discussion by Dr. W. V. Balduf of the subject of parasitism among the insects.

Dr. Gertrude Cassidy presented a note regarding a Java collection of nematodes which is being identified under Dr. Steiner's direction. The nematodes were obtained from the roots of two young coffee plants and were collected by the Baermann method. The total number found was 2,794. Of these 45% were of the genus Acrobeles, 20% were of the genus Tylenchus, 10% were of the genus Paratylenchus. Altogether the collection embraces 14 genera and 28 species. Two of the former are new genera and the latter include 18 new species. The list of genera is as follows: Acrobeles, 11 species (9 n. sp.); Diploscapter, 1 species; Dorylaimus, 3 species (1 n. sp.); Cephalobus, 2 n. sp.; Rhabdolaimus, 1 n. sp.;

Axonchium, 1 n. sp.; Paraphelenchus, 1 species; Aphelenchus, 2 n. sp.; Tylenchus, 3 species (1 n. sp.); Plectus, 1 n. sp.; Paratylenchus, 1 species; Achromadora, 1 n. sp.; and two new genera.

Mr. B. G. Chitwood discussed the excretory system of *Rhabdias bufonis* larvae of the parasitic generation. (Details to appear elsewhere.) In discussion, Dr. N. A. Cobb called attention to the double renette cells in many species of nemas and expressed the opinion that the so-called H-shaped excretory system with tubes in the lateral cords is not the primitive or even the typical nemic excretory system. The excretory tubes are occasionally located in the lateral cords because it is a convenient place to "store" them, and not because there is a close physiologic relationship between these two structures. In this connection it was pointed out that a renette or excretory pore is apparently unknown for any species of Dorylaimus. May it not be possible that these structures have escaped detection because they are double and with two pores situated laterally? Dr. Cobb also noted the reduction of the renette in certain carnivorous nemas, citing members of the genera Mononchus and Dorylaimus as examples, and speculated as to whether it might or might not be true that there is a reduction in the nitrogenous excretory matter in carnivorous animals.

Dr. E. B. Cram reported the isopod *Porcellio scaber* as the intermediate host of *Dispharynx spiralis* of birds in this country. A study being conducted in cooperation with the New England Ruffed Grouse Investigation has resulted in experimentally produced infestations with *D. spiralis* in ruffed grouse (*Bonasa umbellus*) and bobwhite quail (*Colinus virginianus*), from the nematode larvae developed experimentally in the isopod.

Dr. William A. Hoffman reported an infection with *Moniliformis moniliformis* as follows: In November a half grown wild rat was observed in the parasitological laboratory of the School of Tropical Medicine, San Juan, P. R. Its easy capture indicated an abnormal condition. At autopsy the small intestine yielded 167 specimens of *Moniliformis moniliformis*, which completely occluded the lumen. The remainder of the alimentary canal was patent. The weakness of the rat apparently resulted from starvation brought about by the abundance of the acanthocephalids. No other parasites were found.

J. R. Christie, *Secretary.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Fifth Annual Meeting, Des Moines, Iowa, December 28 to 31, 1929.

The meeting was interesting and well attended. Special features included the symposium on veterinary parasitology, the demonstration and tea, and the address of the retiring president. At the annual business meeting the preliminary report of the Treasurer for 1929 was read and approved; the final detailed report was referred to the Council for audit.

Back dues paid in 1929	$ 17.00
Dues paid for 1929	429.00
Total dues through 1929	$446.00
Deficit for 1928	2.40
Amount available for 1929	$443.60
Expenditures to December 24, 1929	309.28
Balance on hand for 1929	$134.32
Advance dues	326.00
Total on hand December 24, 1929	$460.32

The report of the Secretary on membership was read and approved:

Status of Members, Dec. 24, 1929	United States	Foreign Countries	China Branch	Total
Members in good standing	402	66	18	486
Members delinquent for 1929	40	10	24	74
Members delinquent for 1928 and 1929	...	..	4	4
Membership to date	442	76	46	584
Members lost previously	68	15	..	83
Total membership since organization	510	91	46	647

On recommendation of the Council the following permanent committee on nomenclature was appointed: G. Steiner, chairman; Paul Bartsch, Eloise Cram, H. J. Van Cleave, and G. R. La Rue.

The Council, as a nominating committee, proposed the following for officers for 1930; they were unanimously elected.

President, W. W. Cort.

Vice President, E. C. Faust.

Secretary-Treasurer, N. R. Stoll.

Council members for four years, W. B. Herms and Benjamin Schwartz.

W. W. Cort, *Secretary-Treasurer.*

BOOK REVIEWS

INSECTS, TICKS, MITES AND VENOMOUS ANIMALS OF MEDICAL AND VETERINARY IMPORTANCE—PART I. MEDICAL. By WALTER SCOTT PATTON and ALWEN M. EVANS. 786 pp., 374 figs. Obtainable only from the Entomological Department, Liverpool School of Tropical Medicine.

The authors have planned a splendid and much needed work especially since Patton and Cragg's Textbook of Medical Entomology which it is specifically planned to replace, is now out of date and out of print though it was the only work in this field at all comprehensive in character. The authors have been outstanding researchers in the general field covered and have contributed a wealth of special contributions as well as various other important texts. This new and more extensive work will meet a need acutely felt by medical officers and students of tropical medicine as well as by laboratory workers. The work of which this volume is only the first part dealing with the medical aspect, is truly a reference manual containing a mass of information which while more than an individual may need at any particular time is sure to be of great service as the field of work develops. It is distinctly unusual in being arranged in the form of a series of items for a university course in tropical medicine. Each "meeting" corresponds to a class period containing either the synopsis of a lecture or of a laboratory period and proceeds regularly through the series of topics considered from the start of the course to the finish. The authors have felt that this plan would serve the best interests not only of those taking post-graduate courses in tropical medicine, but also medical officers approaching this subject for the first time. Much comparative morphology and some phylogeny has been introduced to form a foundation for the comprehension of the systematic part and for accurate work in the field.

The authors regard, however, the most weighty reason for handling the topic in the fashion employed to be found in the isolated position of a medical officer in the tropics without access to literature and frequent need to handle in thorough fashion the unfamiliar phases of the subject. They stress present day ignorance of the part played by the organisms treated in the transmission of a disease and the evident usefulness of such comprehensive studies for arousing interest and aiding research in this field. It would be impossible to review in detail the immense amount of material handled in the compass of this work. The text while detailed and yet much condensed is not lacking in interest. The illustrations are numerous and worthy of the highest praise. Both photographs and drawings are presented in unusually effective form and in themselves make the work a research contribution of exceptional value. Not only have the old time worn illustrations been eliminated but figures used have a real value in their correspondence to actual views under the microscope. It is difficult to overrate the worth of such illustrations. The authors may be commended for the care with which they make acknowledgment for every figure which has been obtained from any outside source.

An unusual feature in connection with the volume is found in the auspices under which it has been produced. It is published directly by the Liverpool School of Tropical Medicine. The price is very reasonable and much below that associated with recent works which have appeared under other auspices. The work of the printers is well done and the only unusual thing in the make-up of the book is the total absence of a table of contents.

The work is dedicated to twelve well-known researchers who died as the result of infections acquired while investigating the diseases transmitted by insects and acari. This honor rôle is most properly placed at the opening of this splendid contribution to the subject. The work deserves wide circulation and it is hoped

that this may be the immediate result of the labor devoted to its preparation. Certainly all investigators will look eagerly for the appearance of the other three parts which are announced for early publication.

THE IMMUNOLOGY OF PARASITIC INFECTIONS. By WILLIAM H. TALIAFERRO. 414 pp., 28 figs. The Century Co., New York, N. Y.

The vast amount of work done in bacteriology on the topic of immunology has led many to think of similar results as possible in the field of animal parasitology. Some have experimented and published their findings in papers widely scattered, yet up to date no one has sought to bring together these data and handle them in connected fashion. Taliaferro's book fills this conspicuous gap and furnishes material for a whole field of new study and experimentation in which he himself has indeed already broken a path by his own investigations.

The introductory chapter discusses what the author regards as the "fundamental concepts of zooparasitism, infection and immunity" and introduces the zoologist to the terminology and viewpoint of bacteriologists and medical workers. Then several chapters are devoted to a detailed record and analysis of experimental work done on animal parasites and results secured by a study of serological reactions, lysins, antibodies, immune serums, cutaneous and other tests, toxins, etc. The concluding chapters are devoted to a discussion of the cellular basis of immunity, its nature and production in infections with protozoon and metazoon parasites, and finally the classification of certain zooparasites on the basis of serological and immunological reactions. The tabulation of results of such tests is a most valuable part of the work and the comprehensive, carefully selected bibliography with which the volume closes will be welcomed by all who make even casual studies in this field. The author points out that the course of infections and the resistance of the host can be studied directly in certain protozoal invasions though this would be impossible with the bacteria and that the worms furnish a far larger mass of parasitic material than is available otherwise. Valuable results, particularly in methods of diagnosis, will follow the prosecution of intensive studies on the immunological aspect of parasitology. The fine appearance of the book is characteristic of the series of which it is a part. The author's style is lucid and the text is conspicuously free from errors. All in all the volume is a most useful and valuable addition to the literature of animal parasitology and will be welcomed also by many outside of that field.

DISEASES TRANSMITTED FROM ANIMALS TO MAN. By THOMAS G. HULL. 352 pp., 29 figs. Charles C. Thomas, Springfield, Ill.

The great variety of ways in which diseases are transmitted from animals to man has been discussed in a multitude of individual contributions dealing with specific types of disease producing organisms or transmitting agents. It has been left for Dr. Hull to bring together in the compass of a single volume a wealth of material which will appeal to many types of workers, but to none more strongly than to students of animal parasitology. Section one deals with the diseases of domestic animals and birds, section two with rodent affections, section three with human diseases spread by animals, section four with animals as passive carriers of disease organisms, and section five with the rôle played by the more common animals in the spread of disease. Each section is accompanied by a brief bibliography which might have been extended to advantage and certainly should have covered original articles rather than have referred so often to brief abstracts. Some unfortunate errors in proof reading and in citation have also crept into these lists. Undoubtedly this is the weakest feature of the book. The introduction of titles surely would have been useful in guiding the reader toward a selection of those items with which he was particularly concerned.

Each of the diseases is handled individually with a brief historical background and an emphasis on epidemiology together with the means for preventing infection.

On the whole the balance of material is good in comparison with the size of the work, although each reader will probably feel that items in which he is particularly interested have been treated too briefly. The last section on the rôle played by various animals in the spread of disease might well have been considerably extended. It hardly represents the present state of knowledge. It is so briefly treated that important items referred to in earlier chapters are not included here. Further, the text is so condensed that some items are easily misunderstood and others actually erroneous; e.g., the statement that two flukes "are natural inhabitants of the cat from which man is infected," whereas in truth no human infection could possibly result from the most intimate and continued contact of man with infected cats. The book is attractively printed. It will be used and appreciated by many who have been looking for some handy presentation of the field.

Professor K. E. Skrjabin and Dr. R. E. C. Schultz have just issued in Moscow under the auspices of the State Medical Publishing Society a text entitled *Helminthoses of Man, The Fundamentals of Medical Helminthology.* The work contains a general section on the problems of parasitology and separate chapters devoted to particular parasites. The text is well illustrated and well indexed; it should be a valuable aid for training workers in this field in which much activity is manifested at present in the S. S. S. R. and in which the senior author especially has achieved outstanding success.

The Biological Institute at São Paulo, Brazil, has issued recently a volume of the *Archives do Instituto Biologico,* a publication of which the initial number was reviewed previously. The present issue is largely devoted to a series of papers on Bovine Salmonellosis and its relations to tick induced diseases. Other important papers of interest in animal parasitology deals with Piroplasma, nematodes parasitic in plants, and some insects that prey upon the disease transmitting Tabanidae. The sixteen separate articles are illustrated by 44 very fine plates.

NOTES

The First International Congress of Microbiology organized by the Société Internationale de Microbiologie will be held in Paris at the Pasteur Institute and in the Palace of Congresses, July 20 to 25, 1930. The honorary president of the Congress is Dr. Roux, and the general secretary for English speaking countries Dr. Harry Platz, Institut Pasteur, Paris. Those desiring to be enrolled as members may forward the fee (100 French francs) to the treasurer, M. Georges Masson, 120 Boulevard St. Germain, Paris (VI^{e}). Conferences and demonstrations in parasitology are announced by Professors Brumpt, Mesnil, Fülleborn, Nuttall, and others.